12/21/01

FIGS 1-6 ← 09/006,678 01/13/98 - CIP

MLV-MCAT
MLV-TVA
HIV-CD4/CXCR4
CCR5

05/20/97 - PROV

MMC

INTE00004-100 **PATENT**

SERIAL NO.: 10/032,311 **FILED: December 21, 2001**

AMENDMENTS TO THE CLAIMS: 1, 2, 5-8

This listing of claims will replace all prior versions, and listings, of claims in the application:

Please amend claim 1. Please cancel claim 50.

RVLPS
VLPS
VIRAL
PSEUDOTYPES
MULTIPLE TRANSMEMBRANE
SEVEN-SPANNING MEMBRANE PLOTS.

MLV - CXCR4
MLV - CCR5
ALTERED TROPISM

Listing of Claims

1. (Currently Amended) An isolated virus-like particle comprising an enveloped virus core said virus like particle further comprising a heterologous multiple membrane spanning protein ~~of interest wherein said protein is not CD63~~.

REAV
SIV
MLV
FIV
HIV

2. (Previously Presented) The virus-like particle of claim 1, wherein said protein is capable of binding with a ligand under conditions wherein said ligand would bind with an otherwise identical protein present on a cell membrane.

Q: DOES VSV G CONTAIN ~~SPAN~~ MULTIPLE MSP?

3-4. (Canceled)

5. (Previously Presented) The isolated virus-like particle of claim 1, wherein said enveloped virus core is derived from a retrovirus.

6. (Previously Presented) The isolated virus-like particle of claim 1, wherein said enveloped virus core is selected from the group consisting of a murine leukemia virus, a human immunodeficiency virus, a rabies virus, a Rous sarcoma virus, and a vesicular stomatitis virus.

BALLIET
1998
MCAT=MMSP?

7. (Previously Presented) The isolated virus-like particle of claim 1, wherein said protein of interest is selected from the group consisting of a G-protein coupled receptor, a transporter protein, and an ion channel protein.

-LAB

8. (Previously Presented) The isolated virus-like particle of claim 1, wherein said protein of interest is selected from the group consisting of CCR5, CXCR4,, MCAT-1, CXCR2, CXCR3, mu-opioid receptor, and KCNH2 potassium channel protein.

CD4=SMSD
NOT MMSD

9. (Withdrawn) A composition comprising the isolated virus-like particle of claim 1 attached to a sensor surface.

10. (Withdrawn) The composition of claim 9, wherein said protein of interest is selected from

ACTUAL EXAMPLES
HIV VLP W/ CD4 + CXCR4/CCR5
MLV W/ TVA (RSV RECEPTOR)
MLV W/ CCR5/CXCR4
MLV W/ NP-1

BACTERIORHODOPSIN
OPSINS RHODOPSIN
PORINS
β-ADRENERGIC RECEPTOR,
MCAT-1 (MLV RECEPTOR, AA TRANSPORTER)

the group consisting of a transport protein, a G-protein coupled receptor, an ion channel protein, a type I membrane protein, and a type II membrane protein.

11. (Withdrawn) The composition of claim 10, wherein said G-protein coupled receptor is selected from the group consisting of a mu-opioid receptor, a CXCR2, CXCR3, CXCR4, a CCR5, a CCR8, a XCR1, and a CX3CR1.

12. (Withdrawn) The composition of claim 10, wherein said ion channel protein is selected from the group consisting of KCNH2 potassium channel protein, Kv1.3 potassium channel protein, and CFTR protein.

13. (Withdrawn) The composition of claim 10, wherein said transporter protein is selected from a group consisting of a glucose transporter protein and an amino acid transporter protein.

14. (canceled)

15. (Withdrawn) The composition of claim 10, wherein said type II membrane protein comprises DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN).

16-17. (canceled)

18. (Withdrawn) The composition of claim 9, wherein said enveloped virus core is derived from a retrovirus.

19. (Withdrawn) The composition of claim 18, wherein said retrovirus is selected from the group consisting of a murine leukemia virus, a human immunodeficiency virus, a rabies virus, a Rous sarcoma virus, and a vesicular stomatitis virus.

20. (Withdrawn) The composition of claim 9, wherein said virus-like particle further comprises a plastic bead core to form a proteoliposome.

21. (Withdrawn) The composition of claim 9, wherein said sensor comprises a microfluidic device.

22. (Withdrawn) The composition of claim 21, wherein said microfluidic device is a biosensor.

23. (Withdrawn) The composition of claim 22, wherein said biosensor is an optical biosensor.

24. (Withdrawn) The composition of claim 23, wherein said optical biosensor measures surface plasmon resonance (SPR).

25. (Withdrawn) The composition of claim 23, wherein said surface is located on a biosensor chip.

26. (Withdrawn) The composition of claim 25, wherein said biosensor chip is selected from the group consisting of a gold coated biosensor chip, a gold and dextran coated biosensor chip, and a derivatized gold biosensor chip.

27. (Withdrawn) A method of assessing the binding interaction of a multiple membrane spanning protein with a ligand, said method comprising

(a) contacting a virus-like particle according to claim 1 comprising said multiple membrane spanning protein with a ligand of said protein, wherein said virus-like particle is attached to a substrate; and

(b) detecting any change in said substrate compared with any change in an otherwise identical substrate wherein said protein present on said virus-like particle is not contacted with said ligand,

wherein detecting a change in said substrate wherein said protein present on said virus-like particle is contacted with said ligand compared with said otherwise identical substrate wherein said protein present on said virus-like particle is not contacted with said ligand assesses said binding interaction of said protein with said ligand.

28. (Withdrawn) The method of claim 27, wherein said detecting in (b) is performed using a microfluidic device and said substrate is a sensor surface.

29. (Withdrawn) The method of claim 28, wherein said microfluidic device is a biosensor device.

30. (Withdrawn) The method of claim 27, wherein said biosensor device comprises a microchannel or a microwell.

31. (Withdrawn) The method of claim 29, wherein said biosensor is an optical biosensor.

32. (Withdrawn) The method of claim 31, wherein said optical biosensor is a surface plasmon resonance biosensor device.

33. (Withdrawn) A method of identifying a potential ligand of a multiple membrane spanning protein, said method comprising

(a) contacting a virus-like particle according to claim 1 comprising said multiple membrane spanning protein with a test ligand; and

(b) comparing said surface comprising said virus-like particle comprising said multiple membrane spanning protein contacted with said test ligand with an otherwise identical surface comprising an otherwise identical virus-like particle comprising a protein not contacted with said test ligand,

wherein a difference between said surface comprising said virus-like particle comprising a protein contacted with said test ligand compared with said otherwise identical surface comprising said otherwise identical virus-like particle comprising said protein not contacted with said test ligand is an indication that said ligand is a potential ligand of said protein.

34. (Withdrawn) The method of claim 33, wherein said comparing in (b) is performed using a microfluidic device.

35. (Withdrawn) The method of claim 34, wherein said microfluidic device is a biosensor device.

36. (Canceled)

37. (Withdrawn) The method of claim 33, wherein said multiple membrane spanning protein is selected from the group consisting of a G-coupled protein receptor (GCPR), a transporter, and an ion channel.

38. (Withdrawn) The method of claim 36, wherein said single membrane spanning protein is selected from the group consisting of a type I membrane protein and a type II membrane protein.

39. (Withdrawn) The method of claim 33, wherein said test ligand is selected from the group consisting of a protein and a chemical compound.

40. (Withdrawn) The method of claim 39, wherein said protein is an antibody.

41. (Withdrawn) A ligand identified by the method of claim 33.

42. (Withdrawn) A method of identifying a compound that affects binding between a ligand and a membrane protein receptor comprising a multiple membrane spanning protein, said method comprising

(a) contacting a virus-like particle according to claim 1 comprising said protein, wherein said virus-like particle is attached to a surface, with a known ligand under conditions wherein said protein specifically binds with said ligand;

(b) contacting said virus-like particle of (a) with a test compound; and

(c) comparing said surface comprising said virus-like particle contacted with said test compound with an otherwise identical surface comprising an otherwise identical virus-like particle not contacted with said test compound,

wherein a difference between said surface comprising said virus-like particle contacted with said test compound compared with said otherwise identical surface comprising said otherwise identical virus-like particle not contacted with said test compound is an indication that said test compound affects between said ligand and said membrane protein receptor.

43. (Withdrawn) A kit for assessing the binding interaction of a membrane spanning protein with a ligand, said kit comprising a virus-like particle of claim 1 comprising a membrane spanning protein and a substrate, said kit further comprising an applicator, and an instructional material for the use thereof.

44. (Withdrawn) The kit of claim 43, said kit further comprising a ligand of said protein.

45. (Withdrawn) A kit for identifying a potential ligand of a multiple_membrane protein, said kit comprising a virus-like particle of claim 1 comprising a membrane protein and a surface, said kit further comprising an applicator, and an instructional material for the use thereof.

46. (Withdrawn) The kit of claim 45, said kit further comprising a test ligand.

47. (Withdrawn) A kit for identifying a compound that affects binding between a ligand and a membrane protein receptor comprising a multiple membrane spanning protein, said kit comprising a virus-like particle of claim 1 comprising a membrane protein and a surface, said kit further comprising an applicator, and an instructional material for the use thereof.

48. (Withdrawn) The kit of claim 47, said kit further comprising a test compound.

49. (Withdrawn) The kit of claim 47, said kit further comprising a known ligand of said membrane protein.

50. (Canceled)

parts of a cell. In addition to compartmentation, cellular processes are regulated by enzyme synthesis and destruction. Often enzymes are synthesized at low rates when the cell has no need for their activities; however, upon increased demands by the cell (for instance, appearance of substrate), new enzyme is synthesized. Later, the pool of enzyme is lowered when levels of substrate decrease or the cell becomes inactive.

SUMMARY Functional Design of Proteins

- The function of nearly all proteins depends on their ability to bind other molecules (ligands). Ligand-binding sites on proteins and the corresponding ligands are chemically and topologically complementary. The affinity of a protein for a particular ligand refers to the strength of binding; its specificity, to the restriction of binding to one or a few preferred ligands.
- Enzymes are catalytic proteins that accelerate the rate of cellular reactions by lowering the activation energy and stabilizing transition-state intermediates.
- Enzyme active sites comprise two functional parts: a substrate-binding region and a catalytic region. The amino acids composing the active site are not necessarily adjacent in the amino acid sequence, but are brought into proximity in the native conformation.
- The kinetics of many enzymes are described by the Michaelis-Menten equation. From plots of reaction rate versus substrate concentration, two characteristic parameters of an enzyme can be determined: the Michaelis constant K_m, a measure of the enzyme's affinity for substrate, and the maximal velocity V_{max} (see Figure 3-26).
- Many multimeric enzymes and other proteins exhibit allostery. In this phenomenon, binding of one ligand molecule (a substrate, activator, or inhibitor) induces a conformational change, or allosteric transition, that alters the protein's activity or affinity for other ligands.
- In multimeric proteins that bind multiple ligands, binding of one ligand molecule may increase or decrease the binding affinity for subsequent ligand molecules. Enzymes that cooperatively bind substrates exhibit sigmoidal kinetics (see Figure 3-29).
- Allosteric mechanisms can act like switches, turning protein activity on and off. Cyclic phosphorylation and dephosphorylation of amino acid side chains can have the same regulatory effect. Proteolytic cleavage irreversibly converts inactive zymogens into active enzymes.

3.4 Membrane Proteins

As we've seen, all antibodies have a similar structure and function; enzymes are structurally varied, but all have a catalytic function. In contrast, although all membrane proteins are located at the membrane, they otherwise are both str turally and functionally diverse. As we noted in Chapte and discuss in more detail in Chapter 5, every biologi membrane has the same basic phospholipid bilayer str ture. Associated with each membrane is a set of membr proteins that enables the membrane to carry out its disti tive activities (Figure 3-32). The complement of proteins tached to a membrane varies depending on cell type subcellular location.

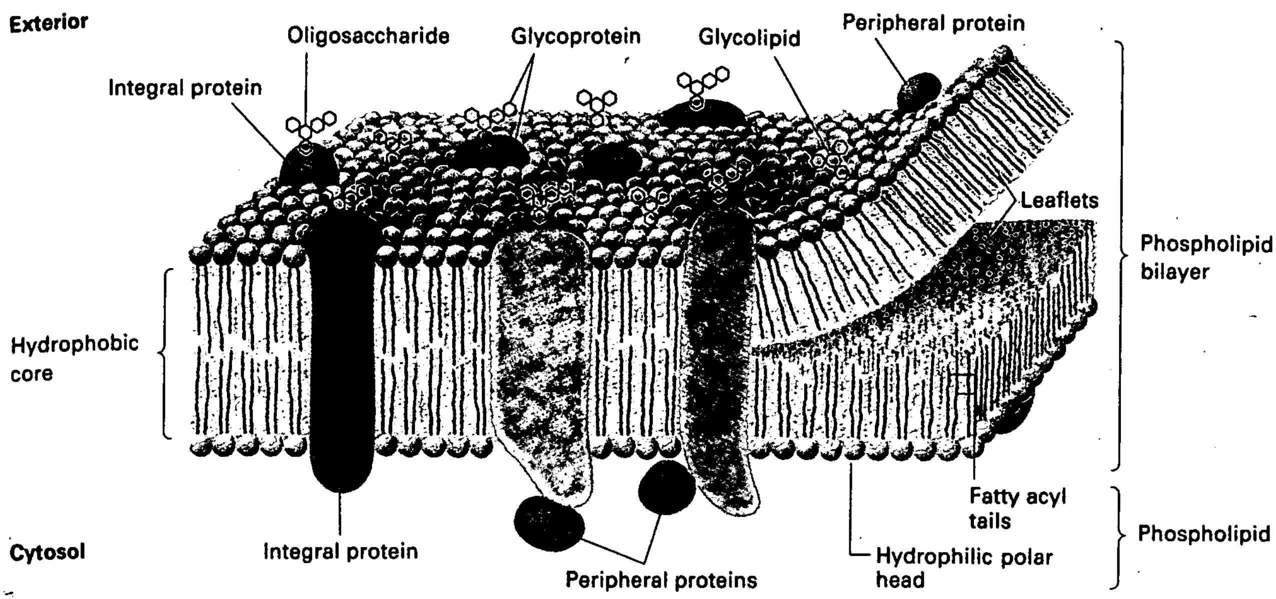
Some proteins are bound only to the membrane surfa whereas others have one region buried within the membr and domains on one or both sides of it. Protein domains the extracellular membrane surface are generally involve cell-cell signaling or interactions. Domains within the me brane, particularly those that form channels and pores, m molecules across the membrane. Domains lying along cytosolic face of the membrane have a wide range of fu tions, from anchoring cytoskeletal proteins to the membr to triggering intracellular signaling pathways. In many ca the function of a membrane protein and the topology of polypeptide chain in the membrane can be predicted ba on its homology with another, well-characterized protein this section, we examine the characteristic structural featu of membrane proteins and some of their basic functic More complete characterization of the structure and fu tion of various types of membrane proteins is presente several later chapters. The synthesis and processing of me brane proteins are discussed in Chapter 17.

Proteins Interact with Membranes in Different Ways

Membrane proteins can be classified into two broad c gories—integral (intrinsic) and peripheral (extrinsic)—ba on the nature of the membrane-protein interactions (see ure 3-32). Most biomembranes contain both types of m brane proteins.

Integral membrane proteins, also called *intrinsic teins,* have one or more segments that are embedded in phospholipid bilayer. Most integral proteins contain resic with hydrophobic side chains that interact with fatty groups of the membrane phospholipids, thus anchoring protein to the membrane. Most integral proteins span entire phospholipid bilayer. These *transmembrane* prot contain one or more membrane-spanning domains as as domains, from four to several hundred residues long, tending into the aqueous medium on each side of the layer. In all the transmembrane proteins examined to d the membrane-spanning domains are α helices or mult β strands. In contrast, some integral proteins are anch to one of the membrane leaflets by covalently bound f acids, as discussed later. In these proteins, the bound f acid is embedded in the membrane, but the polypep chain does not enter the phospholipid bilayer.

Peripheral membrane proteins, or extrinsic proteins, not interact with the hydrophobic core of the phosphol



▲ **FIGURE 3-32 Schematic diagram of typical membrane proteins in a biological membrane.** The phospholipid bilayer, the basic structure of all cellular membranes, consists of two leaflets of phospholipid molecules whose fatty acyl tails form the hydrophobic interior of the bilayer; their polar, hydrophilic head groups line both surfaces. Most integral proteins span the bilayer as shown; a few are tethered to one leaflet by a covalently attached lipid anchor group. Peripheral proteins are primarily associated with the membrane by specific protein-protein interactions. Oligosaccharides bind mainly to membrane proteins; however, some bind to lipids, forming glycolipids.

bilayer. Instead they are usually bound to the membrane indirectly by interactions with integral membrane proteins or directly by interactions with lipid polar head groups. Peripheral proteins localized to the cytosolic face of the plasma membrane include the cytoskeletal proteins spectrin and actin in erythrocytes (Chapter 18) and the enzyme protein kinase C. This enzyme shuttles between the cytosol and the cytosolic face of the plasma membrane and plays a role in signal transduction (Chapter 20). Other peripheral proteins, including certain proteins of the extracellular matrix, are localized to the outer (exoplasmic) surface of the plasma membrane.

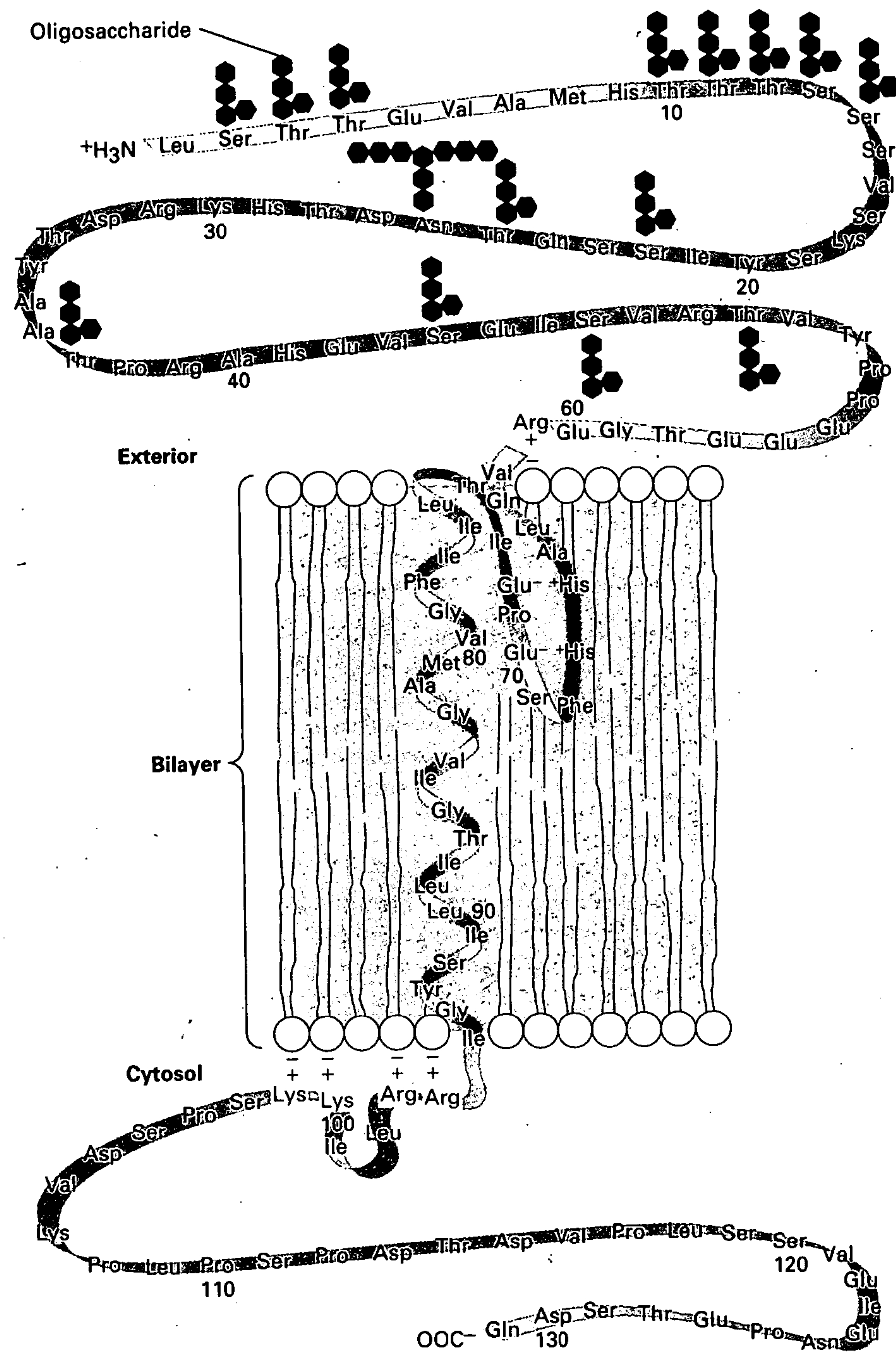
Hydrophobic α Helices in Transmembrane Proteins Are Embedded in the Bilayer

Integral proteins containing membrane-spanning α-helical domains are embedded in membranes by hydrophobic interactions with the lipid interior of the bilayer and probably also by ionic interactions with the polar head groups of the phospholipids. *Glycophorin,* a major erythrocyte membrane protein, exhibits both types of interaction. As shown in Figure 3-33, glycophorin contains a membrane-embedded α helix composed entirely of hydrophobic (or uncharged) amino acids. The predicted length of this α helix (3.75 nm) is just sufficient to span the hydrocarbon core of a phospholipid bilayer. The hydrophobic side chains form van der Waals interactions with the fatty acyl chains and shield the polar carbonyl (C=O) and imino (NH) groups of the peptide bond, which are all hydrogen-bonded to one another. This hydrophobic helix is prevented from slipping across the membrane by a flanking set of positively charged amino acids (lysine and arginine) that are thought to interact with negatively charged phospholipid head groups. In glycophorin, most of these charged residues lie adjacent to the cytosolic leaflet.

Many Integral Proteins Contain Multiple Transmembrane α Helices

Although Figure 3-33 depicts glycophorin as a monomer with a single α helix spanning the bilayer, this protein is present in erythrocyte membranes as a dimer of two identical polypeptide chains. The two membrane-spanning α helices of glycophorin are thought to form a coiled-coil structure (see Figure 3-9a) stabilized by specific interactions between the amino acid side chains at the interface of the two helices. It is now known that many other transmembrane proteins contain two or more membrane-spanning α helices. For instance, the *bacterial photosynthetic reaction center (PRC)* comprises four subunits and several prosthetic groups, including four chlorophyll molecules. In this complex protein, three of the four subunits span the membrane; two of these subunits (L and M) each contain five membrane-spanning α helices (see Figure 16-40).

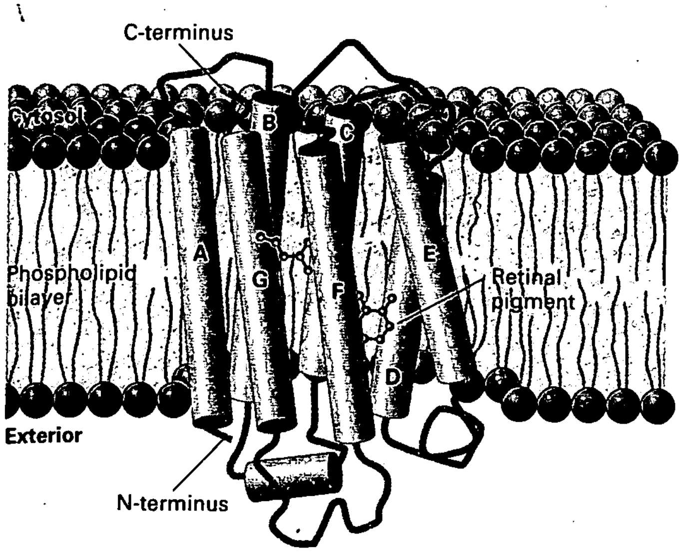
A large and important family of integral proteins is defined by the presence of seven membrane-spanning α helices.



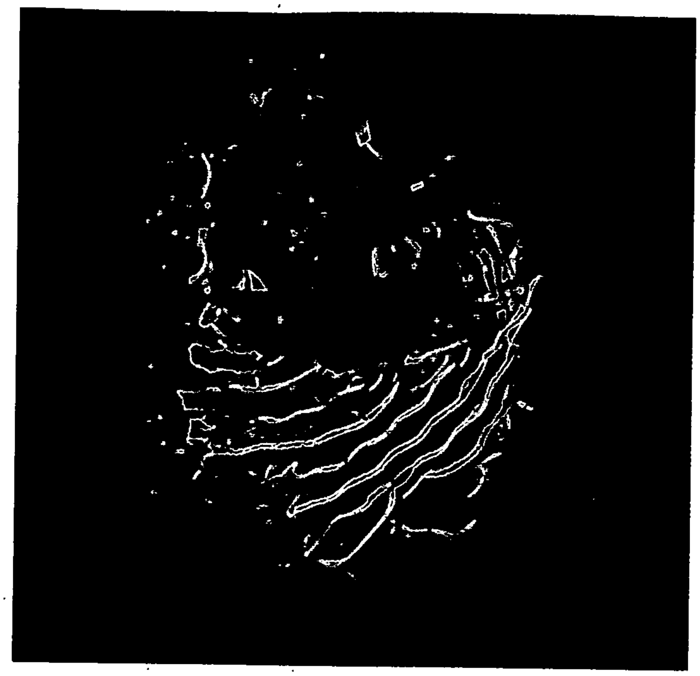
◀ **FIGURE 3-33 Amino acid sequence and transmembrane disposition of glycophorin A from the erythrocyte plasma membrane.** This protein is a homodimer, but only one of its polypeptide chains is shown. Residues 62–95 are buried in the membrane, with the sequence from position 73 through 95 forming an α helix. The ionic interactions shown between positively charged arginine and lysine residues and negatively charged phospholipid head groups in the cytosolic and exoplasmic faces of the membrane are hypothetical. Both the amino-terminal segment of the molecule, located outside the cell, and the carboxy-terminal segment, located inside the cell, are rich in charged residues and polar uncharged residues, making these domains water-soluble. Note the numerous carbohydrate residues attached to amino acids in the exoplasmic domain. [See V. T. Marchesi, H. Furthmayr, and M. Tomita, 1976, *Ann. Rev. Biochem.* **45**:667; A. H. Ross et al., 1982, *J. Biol. Chem.* **257**:4152.]

More than 150 such "seven-spanning" membrane proteins have been identified. This class of integral proteins is typified by *bacteriorhodopsin,* a protein found in a photosynthetic bacterium (Figure 3-34). Absorption of light by the retinal group attached to bacteriorhodopsin causes a conformational change in the protein that results in pumping of protons from the cytosol across the bacterial membrane to the extracellular space. The proton concentration gradient thus generated across the membrane is used to synthesize ATP, as discussed in Chapter 16. Both the overall arrangement of the seven α helices in bacteriorhodopsin and the identity of most of the amino acids can be resolved by computer analysis of micrographs of two-dimensional crystals of the membrane-embedded protein taken at various angles to the electron beam.

Other seven-spanning membrane proteins include the opsins (eye proteins that absorb light), cell-surface receptors for many hormones, and receptors for odorous molecules. Amino acid sequence analysis of these proteins has shown that no amino acids are found in the same position in all of



▲ **FIGURE 3-34 Overall structure of bacteriorhodopsin as deduced from electron diffraction analyses of two-dimensional crystals of the protein in the bacterial membrane.** The seven membrane-spanning α helices are labeled A–G. The retinal pigment is covalently attached to lysine 216 in helix G. The approximate position of the protein in the phospholipid bilayer is indicated. [Adapted from R. Henderson et al., 1990, *J. Mol. Biol.* **213**:899.



▲ **FIGURE 3-35 Model of the three-dimensional structure of a subunit of OmpF, a porin found in the *E. coli* outer membrane.** All porins are trimeric transmembrane proteins. Each subunit is barrel-shaped with β strands forming the wall and a transmembrane pore in the center. [Courtesy of S. Choe.]

them, and only a few residues are conserved in even a substantial number of them. Nonetheless, each of these proteins contains seven stretches of hydrophobic amino acids long enough (>22 amino acids) to span the phospholipid bilayer. Though direct evidence is lacking, it is thought that all of these proteins adopt a conformation in the membrane similar to that of bacteriorhodopsin. This is one of several examples of how investigators can predict the orientation of proteins in a membrane from the amino acid sequence alone.

Multiple β Strands in Porins Form Membrane-Spanning "Barrels"

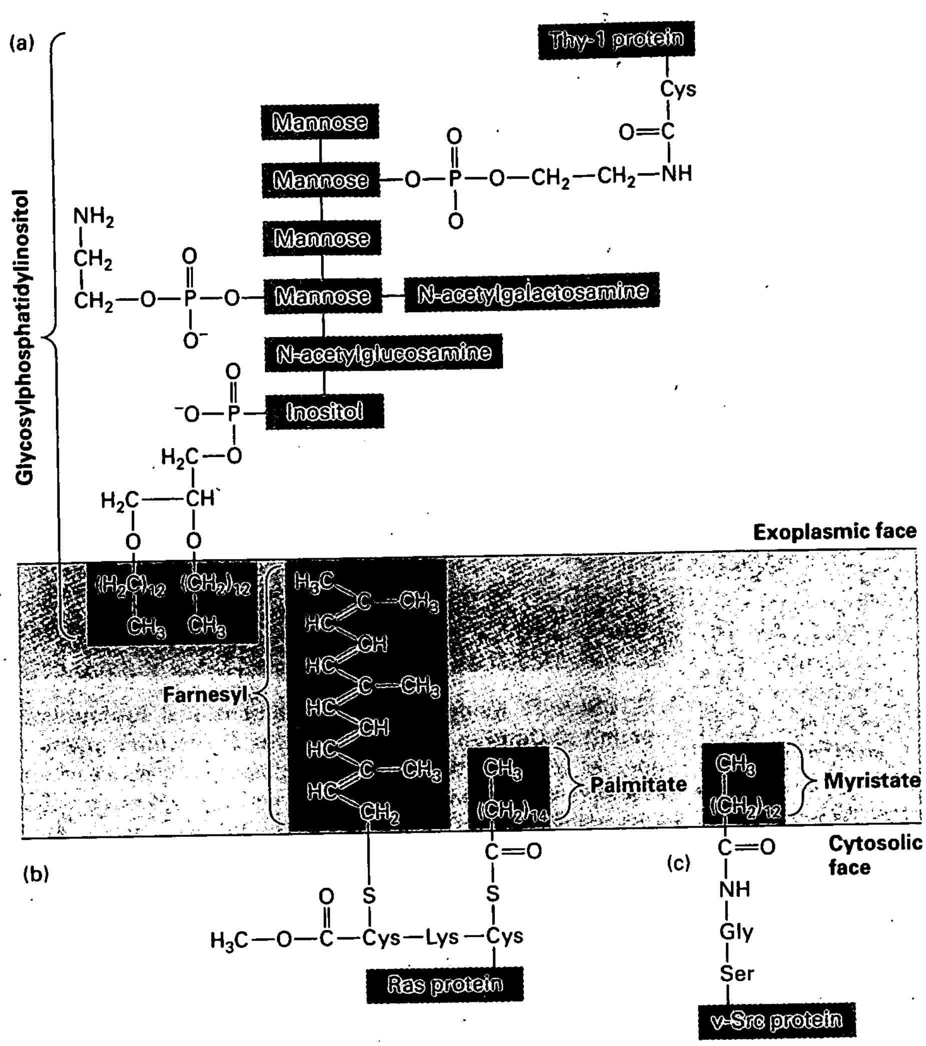
The *porins* are a class of transmembrane proteins whose structure differs radically from that of other integral proteins. Several types of porin are found in the outer membrane of gram-negative bacteria such as *E. coli* (see Figure 1-7a). The outer membrane protects an intestinal bacterium from harmful agents (e.g., antibiotics, bile salts, and proteases) but permits the uptake and disposal of small hydrophilic molecules including nutrients and waste products. The porins in the outer membrane of an *E. coli* cell provide channels for passage of disaccharides, phosphate, and similar molecules.

The amino acid sequences of porins are predominantly polar and contain no long hydrophobic segments typical of integral proteins with α-helical membrane-spanning domains. X-ray crystallography has revealed that porins are trimers of identical subunits. In each subunit 16 β strands form a barrel-shaped structure with a pore in the center (Figure 3-35). As noted earlier, half the amino acid side groups of a β strand point in one direction, and the other half point in the opposite direction (see Figure 3-8). Unlike a typical globular protein, porins have an inside-out arrangement. In a porin monomer, the outward-facing side groups on each of the β strands are hydrophobic and thus can interact with the fatty acyl groups of the membrane lipids or with other porin monomers. The side groups facing the inside of a porin monomer are predominantly hydrophilic; these line the pore through which small water-soluble molecules cross the membrane.

Covalently Attached Hydrocarbon Chains Anchor Some Proteins to the Membrane

In eukaryotic cells, as noted earlier, the polypeptide chain of some integral membrane proteins does not enter the bilayer but rather is anchored in one leaflet by a covalently attached hydrocarbon chain. Several common lipid anchors are shown in Figure 3-36.

Some cell-surface proteins are anchored to the exoplasmic face of the plasma membrane by a complex glycosylated phospholipid that is linked to the C-terminus. A common example of this type of anchor is *glycosylphosphatidylinositol,* which contains two fatty acyl groups, *N*-acetylglucosamine, mannose, and inositol (see Figure 3-36a). Several enzymes,



◀ **FIGURE 3-36 Anchoring of integral proteins to the plasma membrane by membrane-embedded hydrocarbon groups (highlighted in red).** (a) Thy-1 protein and several hydrolytic enzymes are anchored by glycosylphosphatidylinositol. This complex anchor is found only on the exoplasmic face. (b) Cytosolic proteins involved in signaling such as Ras are anchored to the cytosolic face of the membrane through farnesyl and palmitoyl groups. (c) Other cytosolic proteins are associated with the membrane through myristate and similar fatty acids attached to an N-terminal glycine residue.

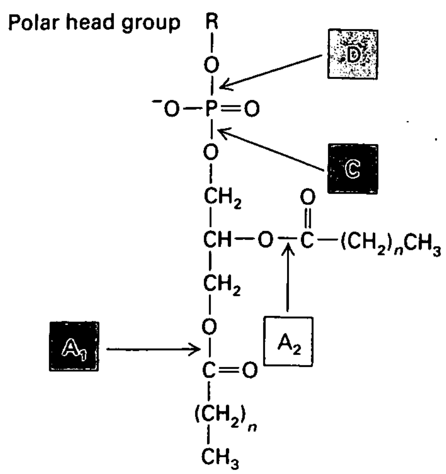
including alkaline phosphatase, fall into this class. Various experiments have shown that the phospholipid anchor is both necessary and sufficient for binding these cell-surface proteins to the membrane. For instance, the enzyme phospholipase C cleaves the phosphate-glycerol bond in phospholipids as well as in glycosylphosphatidylinositol anchors, and treatment of cells with phospholipase C releases glycosylphosphatidylinositol-anchored proteins such as Thy-1 protein and alkaline phosphatase from the cell surface.

Some cytosolic proteins are anchored to the cytosolic face of membranes by a hydrocarbon moiety covalently attached to a cysteine near the C-terminus. The most common anchors are prenyl, farnesyl, and geranylgeranyl groups. These proteins undergo a chemical modification involving several steps. First, the anchor moiety forms a thioether bond with the thiol group of a cysteine that is four residues from the C-terminus of the protein. The modified protein then undergoes proteolysis and methylation; these reactions remove the three terminal residues and add a methyl to the new C-terminus. In some cases, fatty acyl palmitate groups form thioester bonds to nearby cysteine residues, providin additional anchors that are thought to reinforce the attachment of the protein to the membrane (see Figure 3-36b)

In another group of lipid-anchored cytosolic proteins a fatty acyl group (e.g., myristate or palmitate) is linke by an amide bond to the N-terminal glycine residue (se Figure 3-36c). In these proteins, the N-terminal anchor necessary for retention at the membrane and may pla an important role in a membrane-associated function. Fo example, v-Src, a mutant form of a cellular tyrosine kinase is oncogenic and can transform cells only when it retains myristylated N-terminus.

Some Peripheral Proteins Are Soluble Enzymes That Act on Membrane Components

An important group of peripheral membrane proteins a water-soluble enzymes that associate with the polar hea groups of membrane phospholipids. One well-understoo group of such enzymes are the *phospholipases,* which h drolyze various bonds in the head groups of phospholipi-



▲ **FIGURE 3-37 Specificity of cleavage of phospholipids by phospholipases A_1, A_2, C, and D.** Susceptible bonds are shown in red. R denotes the polar group attached to the phosphate, such as choline in phosphatidylcholine (see Figure 5-27a) or inositol in phosphatidylinositol.

(Figure 3-37). These enzymes have an important role in the degradation of damaged or aged cell membranes.

The mechanism of action of phospholipase A_2 illustrates how such water-soluble enzymes can reversibly interact with membranes and catalyze reactions at the interface of an aqueous solution and lipid surface. When this enzyme is in aqueous solution, its Ca^{2+}-containing active site is buried in a channel lined with hydrophobic amino acids. Binding of the enzyme to a phospholipid bilayer induces a small conformational change that fixes the protein to the phospholipid heads and opens the hydrophobic cleft. As a phospholipid molecule moves from the bilayer into the channel, the enzyme-bound Ca^{2+} binds to the phosphate in the head group and positions the ester bond to be cleaved next to the catalytic site.

SUMMARY Membrane Proteins

- Biological membranes usually contain both integral and peripheral membrane proteins (see Figure 3-32).
- Integral membrane proteins include transmembrane proteins and lipid-anchored proteins.
- Two types of membrane-spanning domains are found in transmembrane proteins: one or more α helices or, less commonly, multiple β strands (as in porins). Proteins containing seven membrane-spanning α helices form a major class that includes bacteriorhodopsin and many cell-surface receptors.
- When the polypeptide chain of a transmembrane protein spans the membrane multiple times, the core of the protein generally is hydrophilic, permitting passage of water-soluble molecules, and the surface is hydrophobic, permitting interaction with the interior of the lipid bilayer.
- Amino acid residues modified with long-chain hydrocarbons anchor some integral proteins to one membrane leaflet (see Figure 3-36).
- Peripheral membrane proteins interact with integral membrane proteins or with the polar head groups of membrane phospholipids. They do not enter the hydrophobic core of the membrane.

3.5 Purifying, Detecting, and Characterizing Proteins

A protein must be purified before its structure and the mechanism of its action can be studied. However, because proteins vary in size, charge, and water solubility, no single method can be used to isolate all proteins. To isolate one particular protein from the estimated 10,000 different proteins in a cell is a daunting task that requires methods both for separating proteins and for detecting the presence of specific proteins.

Any molecule, whether protein, carbohydrate, or nucleic acid, can be separated from other molecules based on large differences in some physical characteristic. Although the sequence of amino acids in a protein uniquely determines its function, the most useful physical characteristic for separation of proteins is *size,* defined as either length or mass. In this section, we briefly outline different techniques for separating proteins based on their size and other properties. These techniques also apply to the separation of nucleic acids and other biomolecules. We then consider general methods for detecting, or *assaying,* specific proteins, including the use of radioactive compounds for tracking biological activity. Finally, we discuss several techniques for characterizing a protein's mass, sequence, and three-dimensional structure.

Proteins Can Be Removed from Membranes by Detergents or High-Salt Solutions

Because water-soluble globular proteins have many exposed hydrophilic groups, they maintain their native conformation and remain individually suspended in an aqueous medium when separated from cells. In contrast, when transmembrane proteins are separated from membranes, their exposed hydrophobic regions interact, causing the protein molecules to aggregate and precipitate from aqueous solutions. Such proteins can be solubilized by detergents, which have affinity both for hydrophobic groups and for water.

Detergents are amphipathic molecules that disrupt membranes by intercalating into phospholipid bilayers and solubilizing lipids and proteins. The hydrophobic part of a detergent molecule is attracted to hydrocarbons and mingles with them readily; the hydrophilic part is strongly attracted to water. Some detergents are natural products, but most are synthetic molecules developed for cleaning and for dispersing mixtures of oil and water (Figure 3-38). Ionic detergents, such as sodium deoxycholate and sodium dodecylsulfate

• In mammalian cells, the only integral ER proteins required for translocation of nascent secretory proteins are the SRP receptor, TRAM, and the Sec61 complex.

17.5 Insertion of Membrane Proteins into the ER Membrane

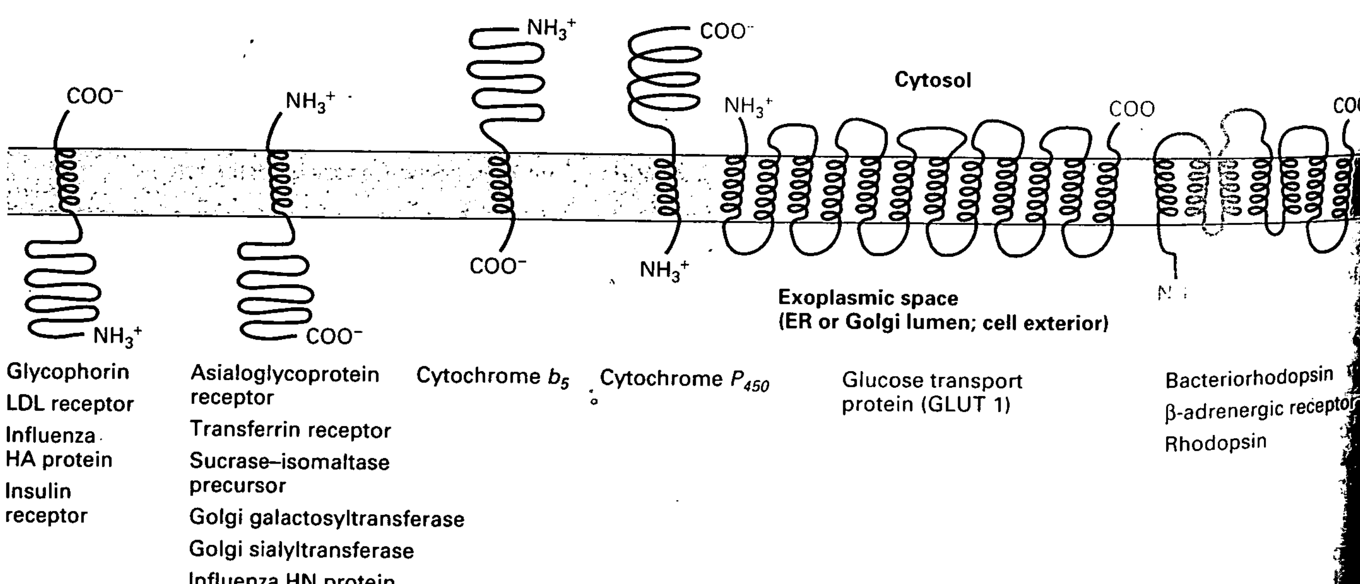
In Chapters 3 and 15 we were introduced to several of the vast array of integral proteins that are present in the plasma membrane and other cellular membranes. Each such protein has a unique orientation with respect to the membrane's phospholipid bilayer (see Figure 3-32). As noted earlier, these membrane glycoproteins are synthesized on the rough ER membrane and remain membrane associated as they move to their final destinations along the same pathway followed by continuously secreted proteins (see Figure 17-13). During this transport, the topology of a membrane protein is preserved; the same segments of the protein, for instance, always face the cytosol. Thus the orientation of these membrane proteins in their final sites is established during biosynthesis on the ER membrane.

As illustrated in Figure 17-21, integral proteins interact with membranes in many different ways. Many such proteins contain a single membrane-spanning segment: a sequence of 20–25 hydrophobic amino acids that forms a transmembrane α helix, anchoring the protein in the phospholipid bilayer. Most of these *single-pass* transmembrane proteins have their hydrophilic N-terminal segment on the exoplasmic face and their hydrophilic C-terminal segment on the cytosolic face; other single-pass proteins have the reverse orientation. Many plasma-membrane proteins have multiple membrane-spanning α-helical segments. Such *multipass* transmembrane proteins include the glucose transporter GLUT1 and numerous G protein–linked cell-surface receptors (Chapter 20). Still other membrane proteins, which lack a hydrophobic membrane-spanning segment, are linked to a glycosylphosphatidylinositol (GPI) anchor that is embedded in the phospholipid bilayer (see Figure 3-36a).

Despite the variation in how integral proteins associate with membranes, insertion of all of them into the ER membrane depends on specific **topogenic sequences**. These sequences of up to 25 amino acids ensure that a protein acquires the proper orientation during its insertion into the ER membrane. In this section, we describe insertion of several types of proteins into the ER membrane to illustrate the function of topogenic sequences.

Most Nominal Cytosolic Transmembrane Proteins Have an N-Terminal Signal Sequence and an Internal Topogenic Sequence

We can begin our discussion with the large class of single-pass transmembrane proteins that have a cytosolic C-terminus



▲ **FIGURE 17-21 Topologies of some integral membrane proteins synthesized on the rough ER.** Segments of the protein chain in the membrane bilayer are shown as transmembrane α helices; the portions outside the membrane are folded. Topogenic sequences in the protein act during biosynthesis to ensure its proper orientation in the ER membrane, which is retained during transport to the cell surface. [See W. Wickner and H. F. Lodish, 1985, *Science* **230**:400; E. Hartmann et al., 1989, *Proc. Nat. Acad. Sci.* **86**:5786; and C. A. Brown and S. D. Black, 1989, *J. Biol. Chem.* **264**:4442.]

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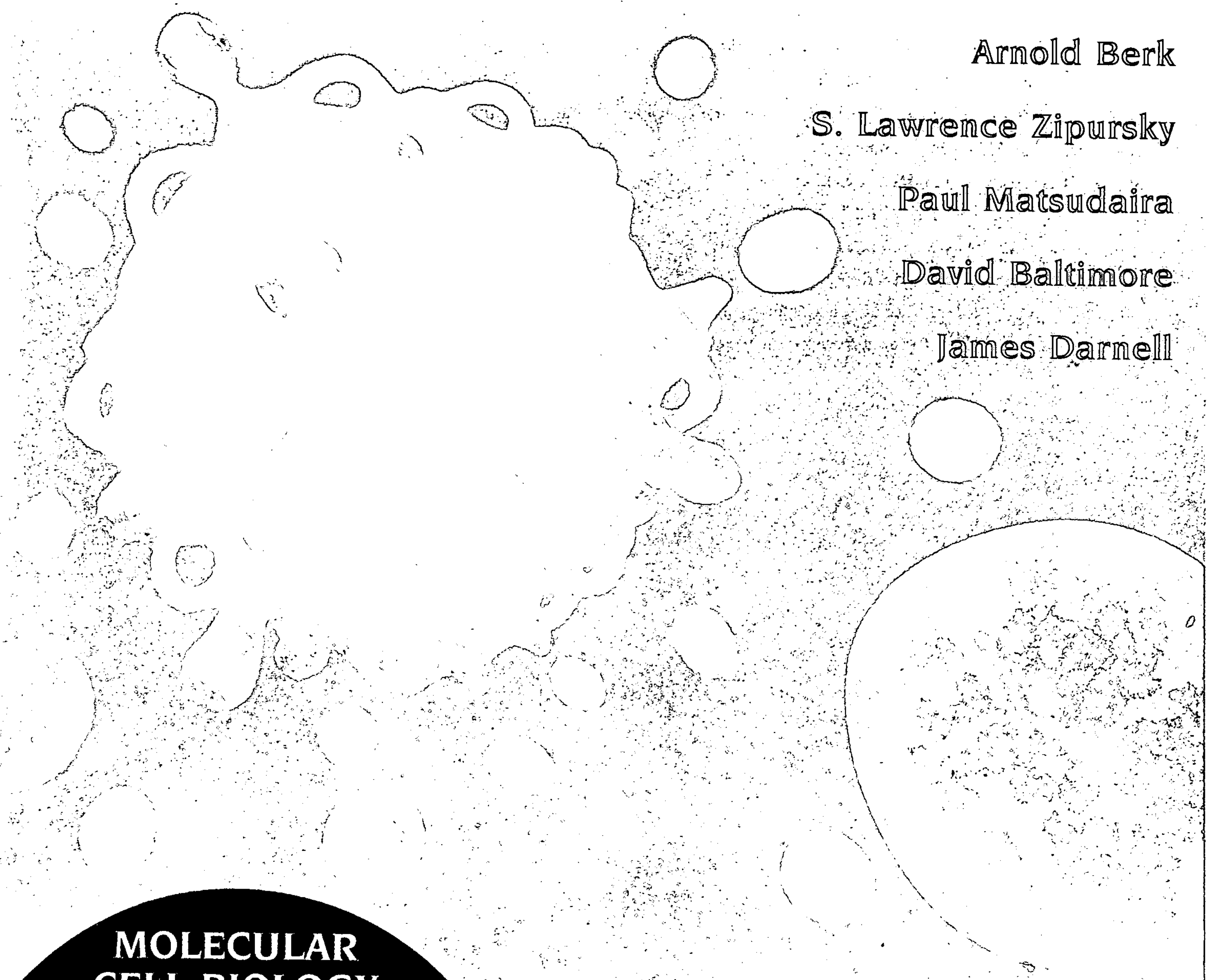

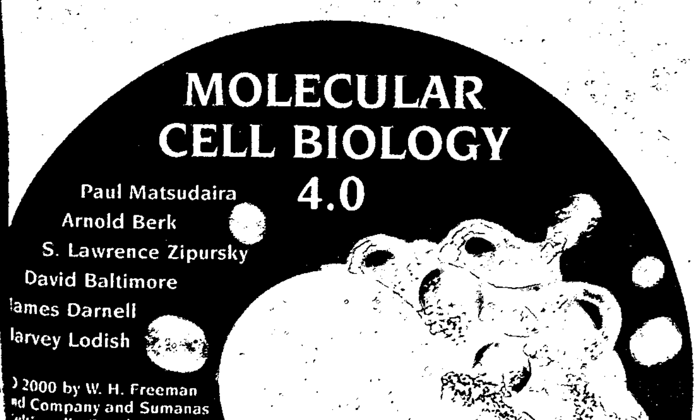

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A Seven-Transmembrane Domain Receptor Involved in Fusion and Entry of T-Cell-Tropic Human Immunodeficiency Virus Type 1 Strains

JOANNE F. BERSON,[1] DEBORAH LONG,[1] BENJAMIN J. DORANZ,[1] JOSEPH RUCKER,[1] FRANK R. JIRIK,[2] AND ROBERT W. DOMS[1]*

Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104,[1] and Biomedical Research Centre and Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3[2]

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Entry of human immunodeficiency virus type 1 (HIV-1) into cells requires binding to CD4 and fusion with a cellular membrane. Fusion does not occur in most nonhuman cells even when they express human CD4, indicating that one or more human accessory factors are required for virus infection. Recently, a seven-transmembrane domain protein has been shown to serve as an accessory factor for T-cell-tropic (T-tropic) HIV-1 isolates (Y. Feng, C. C. Broder, P. E. Kennedy, and E. A. Berger, Science 272:872–877, 1996). Here we show that expression of this glycoprotein, termed fusin, in murine, feline, simian, and quail cell lines, in conjunction with human CD4, rendered these cells fully permissive for HIV-1 envelope glycoprotein (Env)-mediated membrane fusion. Expression of CD4 or fusin alone did not permit fusion. In addition, introduction of fusin and CD4 into a human cell line, U87MG, that is resistant to HIV-1-induced syncytium formation and to infection by HIV-1 when expressing CD4 alone made this cell line permissive for Env-mediated cell-cell fusion. Fusion was observed only with T-tropic Env proteins. Macrophage-tropic (M-tropic) Env proteins from the SF162, ADA, and Ba-L HIV-1 strains did not fuse with cells expressing fusin and CD4, suggesting that M-tropic viruses utilize an accessory molecule other than fusin. Finally, coexpression of fusin and CD4 made both a murine and feline cell line susceptible to virus infection by T-tropic, but not M-tropic, HIV-1 strains.

The envelope protein (Env) of human immunodeficiency virus type 1 (HIV-1) binds virus to the cell surface via a high-affinity interaction with CD4. A subsequent conformational change results in fusion between the viral envelope and a cellular membrane (for a review, see reference 44). While Env-CD4 interactions have been well characterized, it is clear that binding to CD4 by itself is not sufficient for the subsequent membrane fusion reaction (40). Expression of human CD4 (huCD4) in nonhuman cells generally does not render them susceptible to either virus infection or Env protein-mediated syncytium formation (2, 5, 13, 16, 17, 40). Species restriction to infection is often at the level of virus entry and is unidirectional in nature; human cells bearing CD4 readily form syncytia with nonhuman cells bearing Env (2, 13). Furthermore, there are several examples in which expression of CD4 in human cell lines fails to render them permissive for HIV-1 entry and Env-mediated membrane fusion (12, 13, 30). Finally, HIV-1 strains can sometimes be classified as T-cell-tropic (T-tropic) or macrophage-tropic (M-tropic), depending on their differential abilities to infect these CD4-positive target cells (11, 19, 60). Thus, expression of CD4 does not necessarily lead to membrane fusion.

Cells that are nonpermissive for Env-mediated membrane fusion can be made permissive in several ways. Transient heterokaryons formed between murine cell lines expressing huCD4 and CD4-negative human cells support both HIV-1 entry and syncytium formation (5, 16). This finding indicates that one or more components (or accessory factors) in human cells can, when delivered to nonhuman cells, render them susceptible to HIV-1 infection. That the accessory factor(s) is a component of the plasma membrane was shown by Dragic et al. (17) who found that fusion of human erythrocyte ghosts with nonpermissive cells rendered them susceptible to HIV-1 infection and membrane fusion. While identification of the accessory factor(s) has elicited considerable interest, none of the molecules proposed to serve this role have proven to be required for HIV-1 infection, and some, like CD26, appear to have no role at all in HIV-1 entry (1, 6, 8, 21, 38, 52, 62, 63).

A seven-transmembrane domain protein has recently been reported to serve as an accessory factor for T-tropic, but not M-tropic, HIV-1 strains (23). It has been proposed that the protein, which has been referred to as 7TMS (22), LESTR (39), L5 (32), HM89 (50), and HUMSTR (48), be termed fusin as a consequence of its ability to assist HIV-1 Env-mediated membrane fusion (23). Fusin is widely distributed in human tissues and is expressed at high levels in human peripheral blood mononuclear cells and in a number of hematopoietic cell lines including Jurkat and HL-60 cells (22, 32, 35, 39, 48, 50). Fusin bears approximately 33% homology with members of the CXC and CC chemokine receptor families, though studies with a large number of chemokines have failed to reveal functional interactions with fusin (32, 35, 39, 50). To confirm and extend the findings of Feng et al. (23), we examined the ability of fusin to support Env-mediated syncytium formation and HIV-1 infection in a variety of cell types. Here we show that expression of fusin in a number of nonhuman cell lines, in conjunction with huCD4, renders them fully permissive to T-tropic, but not M-tropic, HIV-1 Env-induced syncytium formation and virus infection. Identification of this accessory factor has important

* Corresponding author. Department of Pathology, University of Pennsylvania, 512 SCL, Philadelphia, PA 19104. Phone: (215) 898-0890. Fax: (215) 573-2078. Electronic mail address: doms@mail.med.upenn.edu.

implications for understanding viral tropism and the mechanisms underlying Env-induced membrane fusion.

MATERIALS AND METHODS

Constructs. Fusin cDNA was cloned from a human fetal spleen cDNA library as previously described (22). In order to facilitate cloning, fusin was subcloned into pSP73 (Promega) by using the upstream *Eco*RI site and the downstream *Kpn*I site provided by the original fusin cloning vector. Fusin was subcloned into the vaccinia virus expression vector pSC59 by using the upstream *Eco*RI site and the downstream *Xba*I site provided by pSP73. Ligation into pSC59 employed *Eco*RI and *Spe*I sites, resulting in the elimination of the *Xba*I and *Spe*I sites. pSC59 provides protein expression driven by the vaccinia virus synthetic early/late promoter as previously described (18). Fusin was also excised with *Eco*RV and *Xho*I and subcloned into the mammalian expression vector pREP8 (Invitrogen) that had been digested with *Hin*dIII, blunted with a Klenow fill-in reaction in order to accommodate the *Eco*RV site, and cut with *Xho*I.

The influenza virus hemagglutinin (HA) affinity tag was introduced at the C terminus of fusin by first creating a unique *Hin*dIII restriction site at the 3' end of the open reading frame, followed by oligonucleotide annealing. PCR primer LESTR2 (GGCCAAGGAAGCTGTTGGCTG) was positioned upstream of the unique *Bam*HI site within fusin, while LESTR1 (AGCTCCCGGGAAGCTTG AGTGAAAACTTGAAGACTCA) was designed to introduce a unique *Hin*dIII restriction site just before the stop codon of fusin while preserving the original amino acid sequence. PCR was performed with primers LESTR1 and LESTR2, and the resulting product was digested with the restriction enzymes *Bam*HI and *Hin*dIII and cloned into the corresponding position in pSP73/Fusin. The resulting plasmid, pSP73/Fusin-Hind, was digested with *Hin*dIII, and the oligonucleotides LESTR5 (AGCTACGATGTTCCGGATTACGCATCTCTTCCCGGG) and LESTR6 (AGCTCCCGGGAAGAGATGCGTAATCCGGAACATCGT) were ligated to introduce the HA tag (YDVPDYASL), which was followed by a stop codon, eliminating the *Hin*dIII site and introducing a new *Xma*I site downstream of the stop codon. The resulting tagged form of fusin (fusin-HA) was subcloned into the vaccinia virus expression vector pSC65 by using the upstream *Bgl*II site provided in pSP73 and the downstream *Xma*I site introduced by the oligonucleotides (18).

Cells. The human cervical carcinoma cell lines HeLa and HeLaT4$^+$ (40) and the human astroglioma cell line U87MG were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID). The feline kidney cell lines CCCS+L− and CCCS+L−CD4 (42) and the murine embryo fibroblast cell line PA317T4 (42) were a kind gift from James Simon, University of Pennsylvania. The Japanese quail fibrosarcoma cell line QT6 (ATCC CRL-1708) was provided by John Balliet, University of Pennsylvania. The following cell lines were obtained from the American Type Culture Collection: NIH 3T3, murine embryo cells (CRL-1658); B-SC-1, African green monkey kidney cells (CCL-26); CV-1, African green monkey kidney cells (CCL-70), and human HuTK− 143B fibroblasts (CRL-8303).

Tissue culture media and supplements were purchased from Life Technologies, Inc., unless otherwise noted. HeLa, HeLaT4$^+$, CCCS+L−, CCCS+L−CD4, PA317T4, NIH 3T3, and HuTK− cell lines were maintained in Dulbecco's modified Eagle medium with a high level of glucose and without L-glutamine, supplemented with 10% fetal bovine serum (Hyclone). CD4 expression was selected for by the addition of 0.5 mg of G418 per ml to the media of the HeLaT4$^+$ and PA317T4 cells and by the addition of 0.2 mg of hygromycin (Boehringer Mannheim Biochemicals) per ml to the medium of the CCCS+L−CD4 cells. B-SC-1, CV-1, and U87MG cells were maintained in minimum essential medium supplemented with 10% iron-enriched bovine calf serum (Intergen). QT6 cells were maintained in medium 199, supplemented with 10% tryptose phosphate broth (Sigma), 5% fetal bovine serum, and 1% chicken serum. All media were supplemented with 2 mM glutamine and penicillin-streptomycin.

Viruses. Christopher Broder of the NIAID kindly provided us with the following panel of recombinant vaccinia viruses encoding the Env proteins of various T- and M-tropic HIV-1 strains (indicated in parentheses) (4): T-tropic recombinants, vSC60 (IIIB), vCB34 (SF2), vCB36 (RF), and vCB41 (Lai, LAV); M-tropic recombinants, vCB32 (SF162), vCB39 (ADA), and vCB43 (Ba-L). The recombinant vaccinia viruses vCB16 (encoding a nonfusogenic, uncleaved IIIB Env) and vSC8 (encoding LacZ) were used as negative controls. Christopher Broder also provided us with the recombinant viruses vCB3 (4) (encoding huCD4), vTF1.1 (encoding the T7 polymerase), and vCB21r (encoding *lacZ* under control of the T7 promoter). The recombinant vaccinia virus vBD4, expressing fusin-HA under control of the vaccinia virus synthetic early/late promoter, was generated by standard techniques utilizing thymidine kinase-negative selection (18). The HIV-1 strains used in this study were the T-tropic virus HxB2 (a kind gift from F. Gonzalez-Scarano, University of Pennsylvania) and the M-tropic virus Ba-L received through the AIDS Research and Reference Reagent Program (25). Virus stocks of HxB2 were prepared in H9 cells cultured in RPMI 1640 with 10% fetal bovine serum. The culture supernatant was collected 7 to 10 days postinfection and filtered through a 0.45-μm-pore-size filter to remove cell debris. Ba-L was amplified in phytohemagglutinin-stimulated peripheral blood mononuclear cells from healthy human volunteers and prepared by Ficoll-Hypaque. The viral supernatant was collected as described above.

Western blotting (immunoblotting) and endoglycosidase F digestion. To express fusin-HA, HuTK− cells were infected with recombinant vaccinia virus vBD4 encoding fusin-HA at a multiplicity of infection of 10. vSC8 vaccinia virus, which possesses all genes in vBD4 except fusin-HA, was used as a control. Cells were harvested 24 h postinfection by lysis with Triton X-100, and cell debris was removed by centrifugation at high speed in a microcentrifuge. The resulting supernatant was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. Samples were mixed with SDS-PAGE urea sample buffer, boiled for 5 min, and loaded on an SDS–10% acrylamide gel containing 4 M urea. The gel was transferred onto a polyvinylidene difluoride membrane (Millipore), blocked with Blotto (phosphate-buffered saline [PBS] with 0.1% Tween 20 and 5% dried milk), and probed with the murine monoclonal antibody 12CA5 directed to the HA tag in Blotto for 2 h at room temperature. Bound antibody was detected by incubation with goat anti-mouse horseradish peroxidase (Boehringer Mannheim) (diluted 1:20,000) in Blotto for 45 min at room temperature followed by chemiluminescent detection of horseradish peroxidase (ECL; Amersham).

For detection of N-linked carbohydrates, 12.5 μl of the cell lysate described above was mixed with 12.5 μl of 0.2 M Na phosphate, pH 8.0 (phosphate buffer) containing 0.1% SDS and 0.1% β-mercaptoethanol. Samples were boiled for 5 min before the addition of 25 μl of phosphate buffer and 6 μl of 10% Triton X-100. Treated samples received 4 U of endoglycosidase F (Boehringer Mannheim), while mock-treated samples did not. Samples were digested overnight at 37°C. Samples were analyzed by SDS-PAGE and Western blotting as described above.

Infection assay for HIV-1 entry. pREP8-LESTR (2 μg of plasmid DNA) was transfected into subconfluent CD4$^+$ feline CCCS+L− cells in 24-well tissue culture plates by using calcium phosphate precipitation. The next day the culture medium was changed, and on day 2 posttransfection, cells were infected with cell-free DNase-treated (50 U/ml for 30 min at room temperature) virus stock. Virus infection was synchronized by a 1.5-h adsorption at 4°C followed by a 30-min incubation at 37°C to allow virus entry. The cells were washed twice with cold PBS, growth medium was replaced, and incubation continued at 37°C. Cell lysates for PCR amplification were prepared at various times postinfection by adding 100 μl of PCR lysing buffer (10 mM Tris-Cl [pH 8.3], 50 mM KCl, 0.01% gelatin, 0.45% Tween 20, 0.45% Nonidet P-40) to approximately 2×10^5 cells. Cell samples were incubated overnight at 56°C and then at 95°C for 10 min to deactivate proteinase K.

Nested PCR was performed to amplify the U3 and U5 long terminal repeat (LTR) DNA sequences, and the products were analyzed by electrophoresis on 1.5% agarose gels, transferred to Hybond N+ (Amersham) and probed with a ^{32}P randomly labeled *Kpn*I 608-bp fragment (containing the LTR sequences) from pHIV1lacZ obtained through the AIDS Research and Reference Reagent Program (41). For the first round of PCR amplification, primers U3$^+$57 (5'-CA CACACAAGGCTACTTCCCTG-3') and U5$^-$596 (5'-GATCTCTAGTTACCA GAGTCAC-3') were used. For the second round, primers U3$^+$127 (5'-TGGA TGGTGCTTCAAGCTAGTA-3') and U5$^-$470 (5'-CAGAGAGACCCAGTAC AGGCAA-3') were used. Species-specific primers CF1 and CF2 (5'-TTTGAC CCCCTGTCATAATATGC-3' and 5'-TATCGGGGTGGAGTCAAGTAC-3', respectively) for feline cells were designed to amplify a 184-bp region of the endogenous feline leukemia virus, CF-14 (3).

Gene reporter fusion assay. To quantitate cell-cell fusion events, we utilized the gene reporter fusion assay described by Nussbaum et al. (51). Effector cells expressed T7 polymerase and Env protein, while target cells expressed β-galactosidase under control of the T7 promoter as well as CD4 and fusin. Generally, all proteins were introduced by infection with recombinant vaccinia viruses, except for those cell lines that constitutively express CD4. Vaccinia virus-encoded proteins were produced by infecting cells at a multiplicity of infection of 10 to 20 for 1.5 to 4 h at 37°C. Fusin was introduced into target cells by transfection 1.5 to 2 h postinfection by the calcium phosphate precipitation method. The inoculum was removed, and the cells were washed twice with PBS and then incubated at 32°C overnight in the presence of rifampin. To initiate fusion, target and effector cells were mixed at different ratios in either 96-well plates (when both effector and target cells were in suspension) or in 24-well plates (when adherent target cells were used) at 37°C in the presence of 1-β-D-arabinofuranosylcytosine. To quantitate fusion at different times after initiation, Nonidet P-40 was added to a final concentration of 0.5%, and aliquots of the cell lysates were monitored for β-galactosidase activity by using the colorimetric assay described by Nussbaum et al. (51). In some cases, β-galactosidase was detected by in situ staining (51), or alternatively, syncytium formation was monitored by fixing the cultures in glutaraldehyde-formaldehyde and staining with a solution of 0.5% methylene blue and 0.17% pararosaniline in methanol.

RESULTS

Expression and detection of fusin. Fusin is a 352-amino-acid protein with approximately 33% homology to members of the CC and CXC chemokine receptor families and is predicted to exhibit the topology shown in Fig. 1. Shared structural features

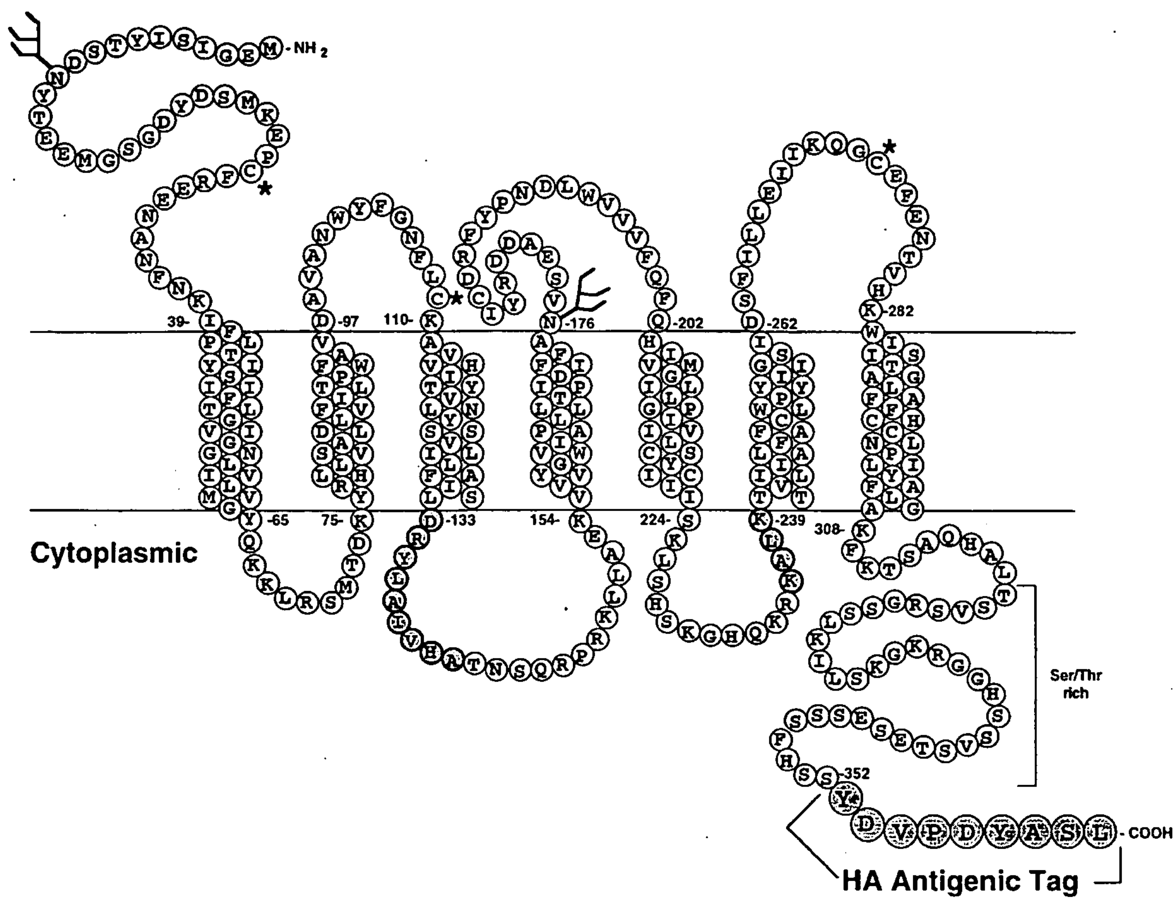

FIG. 1. Schematic representation of fusin. The sequence and proposed membrane topology of fusin are shown. Cys residues in the ectodomain are indicated by an asterisk, and the two potential N-linked carbohydrate addition sites are shown. By analogy with similar receptors, the Cys residues in extracellular loops 1 and 2 are proposed to form a disulfide bond. Conserved regions in cytoplasmic loops characteristic of G-protein-coupled receptors are indicated by shading. An antigenic tag corresponding to a sequence found within influenza virus HA was introduced at the C terminus as indicated.

include seven-transmembrane domains with proline residues in transmembrane domains II, IV, V, VI, and VII, a serine- and threonine-rich C-terminal domain, conserved regions predicted to bind G proteins, two potential N-linked glycosylation sites, and four cysteine residues in the ectodomain (47). By analogy with related molecules, the cysteine residues in extracellular loops 1 and 2 are predicted to form a disulfide bond, as are those in the N-terminal ectodomain and extracellular loop 3 (47).

To express fusin in a variety of cell types, we took advantage of a vaccinia virus vector system. Fusin was cloned into two plasmids, pSC59 and pSC65, thereby placing it under control of the vaccinia virus synthetic early/late promoter (18). These plasmids allowed for transient expression of fusin in two ways: by transfection of the plasmids in conjunction with vaccinia virus infection and by infection with recombinant vaccinia virions. To facilitate detection of fusin, a 9-amino-acid antigenic tag based on the influenza virus HA YDVPDYASL sequence was introduced at the C terminus of fusin. The HA-tagged fusin (fusin-HA) was cloned into pSC65, and a recombinant vaccinia virus was made (vBD4).

To monitor fusin expression, HuTK− cells were infected with vBD4 and incubated overnight. The cells were lysed, and aliquots subjected to SDS-PAGE and Western blotting. Fusin-HA was detected with a monoclonal antibody (12CA5) directed to the HA tag. As shown in Fig. 2 (lanes 1 and 2), fusin-HA was detected by Western blotting as an approximately 50-kDa band. The protein's predicted molecular mass (including the HA tag) is approximately 41 kDa. To determine if fusin contains N-linked carbohydrates, fusin-HA was either mock digested or digested with endoglycosidase F and analyzed by SDS-PAGE and Western blotting. As shown in Fig. 2 (lanes 3 to 5), endoglycosidase F digestion resulted in a 10-kDa shift in mobility from 50 kDa to near its predicted amino acid molecular mass of 41 kDa, indicating that at least one and probably both N-linked consensus sites are utilized (Fig. 1). Identical results were obtained when fusin-HA was immunoprecipitated, digested with endoglycosidase F, and subsequently analyzed by Western blotting (not shown). These results indicate that fusin could be readily expressed and detected by the vaccinia virus expression system and that at least one of two N-linked addition sites is used. Glycosylation of fusin partially confirms the membrane topology depicted in Fig. 1, since there are no potential N-linked glycosylation sites in the proposed cytoplasmic domains.

Fusin supports HIV-1 Env-induced membrane fusion of nonhuman cells. While cells expressing HIV-1 Env form syncytia with most human cells expressing CD4, fusion generally does not occur if the target CD4-bearing cells are nonhuman in origin (2, 5, 13, 16, 17, 40). To determine if fusin could render

FIG. 2. SDS-PAGE analysis of fusin. HuTK− cells were infected with vBD4 (lanes 1, 3, and 4), a recombinant vaccinia virus that expresses fusin with the HA antigenic tag, or with vSC8 (lanes 2 and 5), a virus that does not contain the fusin-HA gene but is otherwise identical to vBD4. After incubation overnight, the cells were lysed and aliquots were subjected to SDS-PAGE in the presence of 4 M urea. Alternatively, aliquots were mock digested (lane 3) or digested with endoglycosidase F (EndoF) (lanes 4 and 5) overnight prior to SDS-PAGE. The proteins were transferred to a polyvinylidene difluoride membrane, and fusin-HA was detected with monoclonal antibody 12CA5. The position of fusin-HA is indicated by the solid arrow, while endoglycosidase F-digested fusin-HA is indicated by the hollow arrow. Samples in lanes 3, 4, and 5 represent 25% of the amount of lysate loaded in lanes 1 and 2. The mobilities of the indicated molecular mass standards (in kilodaltons) are shown to the left of the gel.

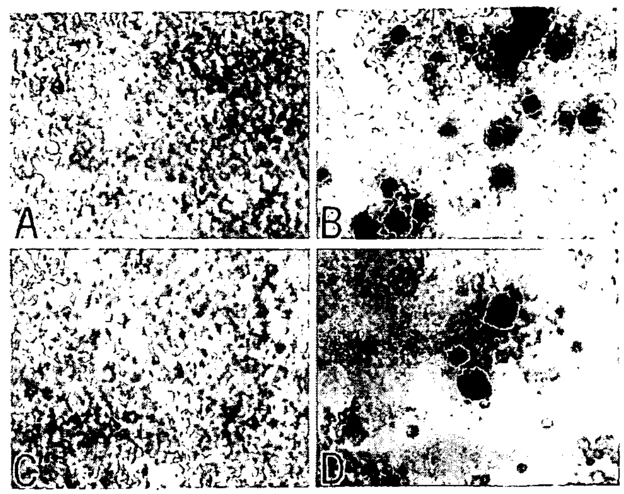

FIG. 3. Fusin supports HIV-1 Env-induced membrane fusion of murine 3T3 cells. HeLa cells infected with vTF1.1 (expressing T7 polymerase) and either vCB16 (expressing a fusion-inactive form of HIV-1 IIIB Env) (A) or vSC60 (expressing HIV-1 IIIB Env) (B, C, and D) were incubated with either HeLa cells (A and B) or murine 3T3 cells (C and D). Both the HeLa and 3T3 cells were infected with vCB21r (encoding *lacZ* under control of the T7 promoter) and vCB3 (expressing huCD4). In addition, the murine cells in panel D were also transfected with pSC59-fusin. After incubation for 8 h, the cells were fixed and stained in situ for β-galactosidase activity.

such cells permissive for HIV-1 Env-mediated membrane fusion, we utilized a vaccinia virus-based gene reporter fusion assay that has been shown to faithfully recapitulate the salient features of HIV-1 Env-mediated membrane fusion (4, 51). In this assay, murine 3T3 cells were either transfected with pSC59-Fusin or mock transfected. The cells were infected with vCB21r, which encodes β-galactosidase under control of the T7 promoter and activates fusin expression. Some cells were also infected with vCB3, a recombinant virus that expresses huCD4. The 3T3 cells were used as targets for HeLa effector cells that expressed the HIV-1 IIIB Env protein and T7 polymerase as a consequence of infection with recombinant vaccinia viruses. Both target and effector cells were infected overnight, after which they were mixed together and incubated at 37°C for up to 8 h. If fusion occurs, the cytoplasmic contents of the target and effector cells mix, leading to β-galactosidase expression (51). Fusion can therefore be monitored in two ways: visually by scoring for syncytium formation (with or without in situ β-galactosidase staining) and biochemically by measuring β-galactosidase activity.

As shown in Fig. 3B, HeLa cells expressing Env readily formed syncytia with HeLa cells expressing CD4. In situ staining for β-galactosidase activity was performed in order to visualize syncytia more easily. Fusion did not occur when a noncleaved, fusion-inactive form of Env was used (Fig. 3A). When HeLa cells expressing fusion-active Env were incubated with murine 3T3 cells expressing huCD4, fusion did not occur, consistent with previous studies (Fig. 3C). However, fusion was readily detected when the target 3T3 cells expressed both CD4 and fusin (Fig. 3D). To quantitate the extent of fusion, various combinations of murine 3T3 target and HeLa effector cells were lysed at different times after mixing, and the amount of β-galactosidase activity was determined by a colorimetric assay. Figure 4 demonstrates that fusion was detected 3 h after mixing and increased with time. Fusion occurred only when cells expressing the fusion-active form of Env were mixed with cells expressing both huCD4 and fusin. Fusion did not occur

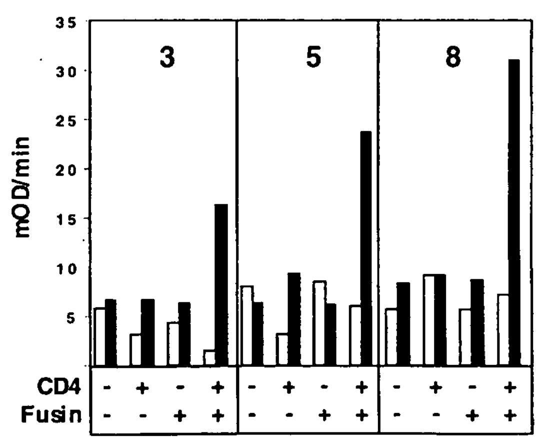

FIG. 4. Time course of fusion. As in Fig. 3, HeLa cells expressing cleaved (fusion-active [black bars]) or uncleaved (fusion inactive [white bars]) IIIB Env and the T7 polymerase were incubated with target 3T3 cells encoding *lacZ* under control of the T7 promoter and expressing the indicated combinations of fusin and huCD4. HuCD4 was introduced by infection with vCB3, while fusin was introduced by transfection. We estimate that 5 to 10% of the target cells were successfully transfected. β-Galactosidase activity was determined at different times after mixing (3, 5, or 8 h, indicated at the top of the panels) and is expressed as milli-optical density units (mOD) per minute.

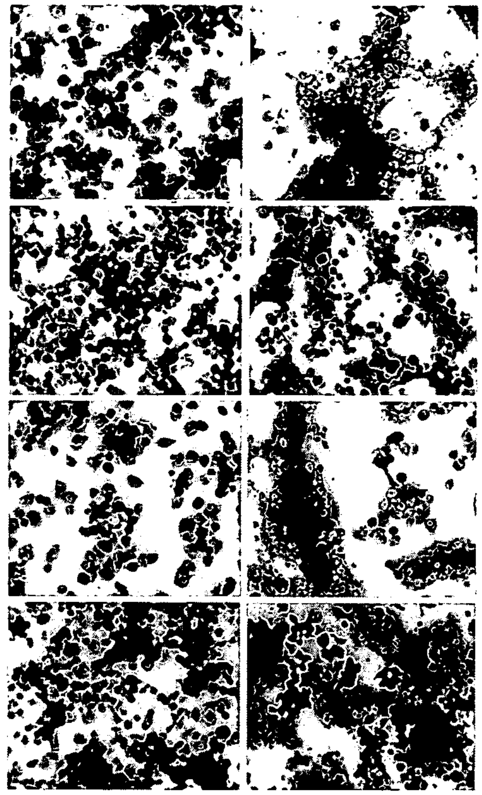

FIG. 5. CD4 and fusin support syncytium formation in different cell types. HeLa cells expressing HIV-1 IIIB Env were mixed with human U87MG, feline CCCS+L−, simian BSC-1, or quail QT6 cells expressing either huCD4 alone or huCD4 and fusin-HA. Both huCD4 and fusin-HA were introduced by use of recombinant vaccinia virus vectors. After 8 h, the cells were fixed and stained with methylene blue.

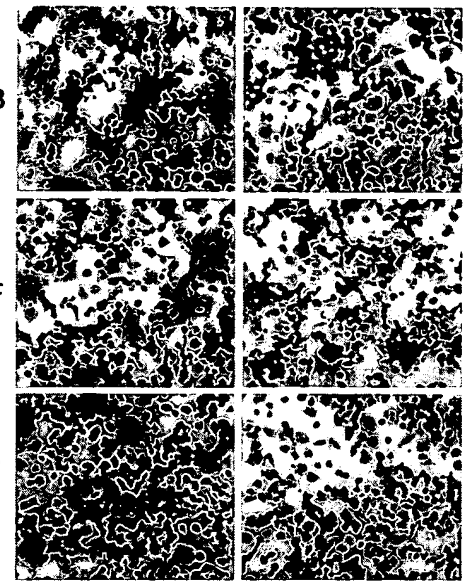

FIG. 6. CD4 and fusin do not support syncytium formation by M-tropic Env proteins. HeLa cells expressing the indicated Env proteins were mixed with feline CCCS+L− cells expressing huCD4 and fusin-HA. The left gels show results with Env proteins from T-tropic viruses, the right gels show results with Env proteins from M-tropic viruses. The cells were fixed and stained with methylene blue 8 h after mixing.

when the target cells expressed either CD4 or fusin alone or when a noncleaved and therefore fusion-inactive form of Env was expressed in the effector cell population (e.g., Fig. 3A).

To determine if the results obtained above were cell type dependent, similar experiments were performed with quail (QT6), feline (CCCS+L−), and simian (BSC-1) cell lines. In addition, a human cell line (U87MG) that is resistant to HIV-induced syncytium formation and infection when expressing CD4 was used (12). huCD4 was introduced into target cells by infection with recombinant vaccinia virus vCB3, while fusin-HA was introduced by infection with vBD4. HeLa cells expressing the HIV-1 IIIB Env protein were used as effector cells. Target and effector cells were incubated together for 8 h, after which the cells were fixed and stained with methylene blue. As shown in Fig. 5, none of the target cells supported syncytium formation when expressing CD4 alone. However, large syncytia formed when both CD4 and fusin were expressed in the target cells. These findings show that coexpression of fusin-HA and huCD4 renders a number of nonhuman cell lines susceptible to HIV-1 Env-induced membrane fusion.

Fusin supports fusion of T-tropic, but not M-tropic, Env proteins. To determine if fusin could serve as an accessory factor for both T- and M-tropic HIV-1 strains, feline CCCS+L− cells expressing huCD4 and fusin were mixed with HeLa effector cells infected with recombinant vaccinia virus vectors that express the Env proteins of the T-tropic strains IIIB, LAV, and RF or the M-tropic strains ADA, Ba-L, and SF-162. The Env proteins expressed by these recombinant viruses have previously been shown to be fusion active (4). As shown in Fig. 6, fusion was readily observed when the effector cells expressed T-tropic Env proteins. Fusion was not observed with the ADA, Ba-L, and SF-162 Env proteins. Thus, while fusin supported fusion by T-tropic HIV-1 strains, it failed to support fusion of the three M-tropic strains tested here.

Fusin supports infection of T-tropic HIV-1 strains. To determine if fusin could render CD4-positive nonhuman cells permissive for HIV-1 infection, we utilized a PCR-based entry assay. $CD4^+$ feline cells (CCCS+L−CD4) were either transfected with pREP8-fusin DNA or mock transfected 48 h prior to virus challenge. At the indicated times postinfection, cell lysates were prepared and subjected to nested PCR analysis using HIV-1 U3 and U5 LTR-specific primers as described in Materials and Methods. An intense band of the predicted size corresponding to the LTR DNA sequence was evident only after PCR amplification of cell lysates prepared from feline cells expressing both fusin and huCD4 and infected with a T-tropic HIV-1 strain, IIIB (HxB2 clone [Fig. 7A]). The very weak band that was sometimes detected for HxB2-infected CD4-positive feline cells not expressing fusin declined over time (Fig. 7A) and so likely represents residual virus inoculum. In contrast, infection of feline cells expressing both fusin and huCD4 with the M-tropic HIV-1 strain Ba-L either resulted in no PCR product or only a very faint band (Fig. 7A). Similar results were obtained with murine PA317-T4 cells (not shown). Amplification with feline specific primers yielded bands of equivalent intensity in all lanes (Fig. 7B), indicating that those samples with weak or no detectable HIV-1-specific sequences contained equal amounts of DNA.

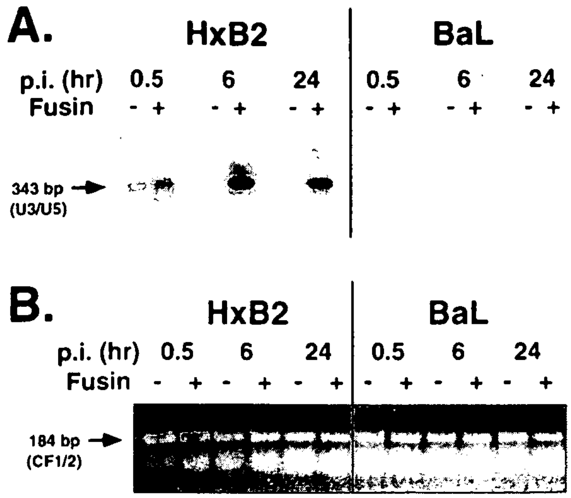

FIG. 7. Fusin supports virus entry. Feline CCCS+L− cells, which constitutively express huCD4, were transfected with pREP8-LESTR as indicated. Two days later, cells were infected with either HIV-1 HxB2 (T-tropic) or Ba-L (M-tropic). Cell lysates were prepared at the indicated times postinfection (p.i.), and PCR amplification of viral U3 and U5 LTR DNA sequences was performed (A). Species-specific primers were used to amplify a 184-bp region of the endogenous feline leukemia virus CF-14 to ensure that equivalent amounts of DNA were used in each sample (B).

DISCUSSION

The entry of HIV-1 into cells is a critical step in the infectious cycle and an important determinant of viral tropism. Binding to a cell surface receptor, such as CD4, must be followed by a conformational change in the Env protein that leads to fusion between the viral envelope and a host cell membrane (for a review, see reference 44). This conformational change is believed to result in the exposure of the N-terminal fusion peptide in the gp41 subunit. While CD4 binding has been shown to induce structural alterations in Env (59), it is clear that CD4 binding in and of itself is not sufficient to trigger the entire sequence of events that lead to membrane fusion and subsequent viral entry. As a consequence, it has been suspected that additional cofactors resident in the plasma membrane of the target cell participate in this process. A number of molecules have been proposed to serve as accessory factors for HIV-1, including LFA (33), CD7 (58), and CD26 (7). While some of these molecules may enhance syncytium formation under certain circumstances, none has proven to be required for infection or cell-cell fusion. In the case of CD26, many investigators have reported that it does not play a role either in virus infection or syncytium formation (1, 6, 8, 21, 38, 52, 62, 63).

In this report, we have shown that fusin, a seven-transmembrane domain glycoprotein, can serve as an accessory factor for a number of T-tropic HIV-1 strains, in agreement with Feng et al. (23). Fusin fulfills the requirements of an accessory factor in that it rendered a number of murine, feline, simian, and quail cell lines, as well as a human cell line resistant to HIV-1 entry, fully permissive for HIV-1 Env-mediated syncytium formation. When fusin was introduced into target cells by infection with a recombinant vaccinia virus rather than by transfection such that all target cells expressed both the cofactor and huCD4, fusion was greatly enhanced, with most cells forming syncytia. Specificity was confirmed in all cases by the finding that fusion occurred only when the effector cells expressed fusion-active Env and when the target cells expressed both fusin and huCD4. In agreement with Feng et al., we found that HIV-1 IIIB could infect cells that coexpressed huCD4 and fusin (23). In addition, we extended their findings by showing that fusin failed to support infection by an M-tropic HIV-1 strain. Taken together, these findings suggest that fusin can serve as a cofactor for T-tropic, but not M-tropic, HIV-1 strains for both Env-mediated syncytium formation and virus infection. A larger panel of virus strains, including viruses from different clades, will have to be examined in order to determine the full range of virus types that can utilize fusin as an entry cofactor.

Fusin is a 352-amino-acid protein that is predicted to contain seven transmembrane domains (22, 32, 35, 39, 50). It is most closely related (approximately 33% homology) to the CXC and CC chemokine receptors, though attempts to demonstrate chemokine binding to fusin have not been successful (32, 35, 39, 50). Thus, fusin is an orphan receptor with no known ligand or function. On the basis of its sequence, its homology with better-characterized receptors, and the fact that one or both of the N-linked glycosylation sites are utilized (Fig. 2), it is likely that fusin exhibits the topology depicted in Fig. 1. Fusin contains an acidic N-terminal ectodomain segment that in other chemokine receptors has been implicated in ligand binding (14, 28, 31). The transmembrane domains are notable in that several contain proline residues, and the intracellular loops contain highly conserved motifs characteristic of G-protein-coupled receptors. The four cysteine residues in the ectodomain of fusin include two in the first and second extracellular loops that are highly conserved and are predicted to form a disulfide bond (61). The presence of an N-linked glycosylation site in the amino-terminal domain is also a common structural motif, and it is utilized in a number of other receptors (47). Finally, the C-terminal domain of fusin, like the chemokine receptors, is rich in serine and threonine residues that could be phosphorylated following ligand binding.

Fusin has been cloned from human monocyte, fetal brain, lung, spleen, and peripheral blood mononuclear cell libraries (22, 32, 35, 39, 50). A homolog isolated from bovine brain bears 93% homology with human fusin (56). Northern (RNA) blot analyses show that fusin is expressed at high levels in B- and T-cell lines, in cell lines derived from the monocytic lineage, and from human peripheral blood lymphocytes, monocytes, and neutrophils (22, 35, 39, 50). High levels of expression are seen in heart and brain tissue, with intermediate levels in colon and liver tissue (22). It is also expressed in HeLa cells (50), which is significant since HeLa cells have been shown to contain the accessory factor for T-tropic viruses (5, 16). Human erythrocytes have also been shown to contain an HIV-1 cofactor, since fusion of human erythrocyte ghosts with murine cells expressing huCD4 makes these cells susceptible for HIV-1 Env-mediated syncytium formation (17). While it is not known if fusin is present in the erythrocyte membrane, it is interesting to note that the Duffy blood group antigen, a seven-transmembrane receptor that bears approximately 20% homology to fusin, is present at relatively high levels and has been shown to bind to RANTES, interleukin-8, and MCP-1 (10, 49, 53). Whether the Duffy antigen can serve as an HIV-1 cofactor is currently being examined. Human cell lines in which fusin mRNA has not been detected include the neuroblastoma cell lines SK-N-MC and LAN5. It is interesting to note that expression of CD4 in SK-N-MC cells does not render them permissive for HIV-1 Env-mediated syncytium formation (39a). Thus, the distribution of fusin is largely consistent with its role as a T-tropic accessory factor—it is present in cells that are permissive for virus infection or membrane fusion, and it is absent in at least one human cell line that is not.

How might fusin participate in HIV-1 entry? The most obvious possibility is that it plays a role similar to that of other

viral accessory factors and binds directly to the Env protein, leading to conformational changes that lead to membrane fusion. The Semliki Forest virus, for example, binds to an as yet unidentified receptor on the cell surface. After the virus is internalized and delivered to endosomes, the acid pH triggers a conformational change in the virus spike protein that enables it to bind to cholesterol (for a review, see reference 36). Cholesterol binding, along with the presence of trace quantities of sphingolipids in the target membrane, is required for the subsequent membrane fusion reaction (36). Insect cells that lack cholesterol are not permissive for Semliki Forest virus infection, but introduction of cholesterol makes them fully susceptible (54). For HIV-1, binding to CD4 is known to trigger conformational changes in Env, but these changes are not sufficient for fusion. However, CD4 binding may allow subsequent binding of Env to fusin, which may then lead to conformational changes that result in exposure of the fusion peptide and membrane fusion. Support for this hypothesis comes from the work of Golding and coworkers, who found that the phorbol ester myristate acetate (PMA) can down-regulate CD4, but not a truncated version of CD4 that lacks its cytoplasmic domain (26, 27). However, PMA-induced down-regulation of the truncated CD4 did occur when cells were incubated with soluble gp120 prior to the addition of PMA. Importantly, this did not occur when tailless CD4 was expressed in nonhuman cell lines. These findings suggest that gp120 binding to CD4 induces conformational changes in either gp120 or CD4 that lead to complex formation with the accessory factor, which itself is down-regulated by PMA (26, 27). Alternatively, fusin may affect the way in which CD4 is presented on the cell surface by affecting CD4 conformation or by leading to patching, since multimeric CD4 binding may be required for fusion to occur (37, 45). The identification of fusin as an accessory factor for the HIV-1 fusion reaction will make it possible to test these and other hypotheses.

While fusin supports T-tropic HIV-1 Env-mediated membrane fusion, it does not appear to serve as an accessory factor for M-tropic HIV-1 strains. Given the similarities between T- and M-tropic Env proteins, it is reasonable to suspect that M-tropic viruses utilize a structurally related molecule. Fusin shares considerable homology with both the CC and CXC chemokine receptors. Two CXC chemokine receptors have been identified, one of which binds interleukin-8, while the other binds interleukin-8 and other CXC chemokines (34, 46). Thus far, four CC chemokine receptors have been identified. Of these, CKR-1, CKR-4, and CKR-5 bind to RANTES, MIP-1α, and MIP-1β, while CKR-2 binds to MCP-1 and MCP-3 (9, 24, 43, 48, 55, 57, 64). Recently, it has been shown that RANTES, MIP-1α, and MIP-1β are the major HIV-1 suppressive factors secreted by $CD8^+$ T cells (15). If certain HIV-1 strains can utilize one or more receptors for these CC chemokines, then the antiviral effects of these chemokines may be mediated in part by the blockade or the down-regulation of the chemokine receptor.

HIV-1, HIV-2, and simian immunodeficiency virus isolates are notable for their diverse cellular tropisms. While some virus strains preferentially infect T cells, others infect macrophages, while others are dual tropic and infect both. Furthermore, certain HIV-1 and HIV-2 strains can infect CD4-negative cells, albeit inefficiently (20, 29). While HIV-1 generally is unable to enter nonhuman cells bearing huCD4, this species restriction is not as strict for HIV-2 and simian immunodeficiency virus (13, 42). Cellular tropism is often determined at the level of virus entry, and evidence has accumulated that the presence or absence of cell-specific accessory factors or cofactors determines whether or not a given virus strain can enter a given CD4-positive cell type. The identification of fusin as a cofactor for a number of T-tropic, but not M-tropic, Env proteins is an important step in understanding viral tropism at the molecular level. Given the diversity of Env protein sequences from different viral strains and the large number of seven-transmembrane domain proteins that bear significant homology to fusin, it will not be surprising if other proteins in this class participate in the entry of different HIV-1, HIV-2, and simian immunodeficiency strains.

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A Dual-Tropic Primary HIV-1 Isolate That Uses Fusin and the β-Chemokine Receptors CKR-5, CKR-3, and CKR-2b as Fusion Cofactors

Benjamin J. Doranz,* Joseph Rucker,* Yanjie Yi,†
Robert J. Smyth,† Michel Samson,‡
Stephen C. Peiper,§ Marc Parmentier,‡
Ronald G. Collman,† and Robert W. Doms*
*Department of Pathology and Laboratory Medicine
†Pulmonary and Critical Care Division
Department of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania 19104
‡IRIBHN
Université Libre de Bruxelles
B-1070 Bruxelles
Belgium
§Department of Pathology
James Graham Brown Cancer Center
University of Louisville
Louisville, Kentucky 40202

Summary

Here, we show that the β-chemokine receptor CKR-5 serves as a cofactor for M-tropic HIV viruses. Expression of CKR-5 with CD4 enables nonpermissive cells to form syncytia with cells expressing M-tropic, but not T-tropic, HIV-1 env proteins. Expression of CKR-5 and CD4 enables entry of a M-tropic, but not a T-tropic, virus strain. A dual-tropic primary HIV-1 isolate (89.6) utilizes both Fusin and CKR-5 as entry cofactors. Cells expressing the 89.6 env protein form syncytia with QT6 cells expressing CD4 and either Fusin or CKR-5. The β-chemokine receptors CKR-3 and CKR-2b support HIV-1 89.6 env-mediated syncytia formation but do not support fusion by any of the T-tropic or M-tropic strains tested. Our results suggest that the T-tropic viruses characteristic of disease progression may evolve from purely M-tropic viruses prevalent early in virus infection through changes in the env protein that enable the virus to use multiple entry cofactors.

Introduction

Human immunodeficiency virus type 1 (HIV-1) isolates exhibit marked differences in their abilities to infect CD4-positive cells. While all strains infect primary CD4+ T lymphocytes, most primary isolates from patients also infect primary macrophages (M-tropic), are typically nonsyncytium inducing (NSI), and fail to infect CD4+ transformed cell lines (Cheng-Mayer et al., 1988). Other isolates replicate well in CD4+ cell lines (T-tropic) and are syncytium-inducing (SI) but fail to infect macrophages. Changes in the biological properties of sequential viral isolates from infected individuals have been shown to correlate with disease progression. Thus, most viruses isolated from individuals shortly after seroconversion and during the asymptomatic phase of infection exhibit M-tropic NSI phenotypes (Conner and Ho, 1994; Conner et al., 1993; Roos et al., 1992; Schuitemaker et al., 1991, 1992; Tersmette et al., 1988, 1989a; Zho et al., 1993). With time, viruses emerge in many individuals that have T-tropic SI characteristics as well as increased replicative capacity. The appearance of T-tropic SI viruses correlates with progressive CD4+ lymphocyte decline and the development of AIDS (Tersmette et al., 1988, 1989a, 1989b). Dual-tropic viruses that retain the ability to replicate efficiently in macrophages in spite of having acquired SI characteristics have been described and may represent an important transitional phenotype leading to the T-tropic SI viruses associated with disease progression (Collman et al., 1992; Shibata et al., 1995).

In order to study the selective pressures that drive the transition from M- to T-tropism, the underlying source of permissiveness for M- and T-tropic viruses must be understood. HIV-1 cell tropism is determined largely at the level of virus entry and has been mapped to the envelope (env) protein, specifically to regions in the gp120 subunit that include the V3 loop (Cheng-Mayer et al., 1991; Hwang et al., 1991; Kim et al., 1995; Liu et al., 1990; O'Brien et al., 1990; Shioda et al., 1991; Westervelt et al., 1991, 1992). In order for HIV-1 to infect lymphocytes or macrophages, the env protein must bind to the primary viral receptor, CD4. While CD4 binding results in conformational changes in env (Sattentau and Moore, 1991), these are not sufficient to elicit the env-mediated membrane fusion reaction that is a prerequisite for virus infection. Rather, one or more cofactors are required in conjunction with CD4 for fusion to occur. The cofactor requirement is most clearly demonstrated by studies showing that expression of human CD4 (huCD4) in most nonhuman cells (and some human cell lines) fails to make them permissive for virus infection or env-mediated syncytia formation (Ashorn et al., 1990; Broder and Berger, 1995; Broder et al., 1993; Chesebro et al., 1990; Clapham et al., 1991; Dragic et al., 1992, 1995; Harrington and Geballe, 1993; Maddon et al., 1986). Nonpermissive CD4-positive cells can be made permissive for env-mediated membrane fusion and virus infection by transient heterokaryon formation with HeLa cells, indicating that one or more components (or cofactors) in HeLa cells can, when delivered to nonhuman cells, render the nonpermissive cells susceptible to HIV-1 infection (Broder et al., 1993; Dragic et al., 1992).

Recently, a seven-transmembrane domain protein, termed Fusin, has been shown to serve as a cofactor for T cell–tropic HIV-1 strains (Berson et al., 1996; Feng et al., 1996). Fusin exhibits approximately 30% homology with members of both the α and β-chemokine receptor families, though Fusin has not been shown to bind chemokines itself (Herzog et al., 1993; Jazin et al., 1993; Loetscher et al., 1994; Nomura et al., 1993). Expression of Fusin with huCD4 renders otherwise nonpermissive cell lines susceptible to env-mediated syncytia formation and virus infection (Berson et al., 1996; Feng et al., 1996). However, Fusin does not appear to be utilized as a cofactor by primary M-tropic NSI virus strains. Because of the similarity between Fusin and the chemokine receptors and the fact that the β-chemokines RANTES,

MIP-1α, and MIP-1β are the major HIV-1 suppressive factors secreted by CD8+ T cells (Cocchi et al., 1995; Paxton et al., 1996), we examined the ability of several chemokine receptors to function as cofactors for primary NSI viruses. We found that the β-chemokine receptor CKR-5, which shares 30% amino acid homology with Fusin and has been shown to bind RANTES, MIP-1α, and MIP-1β (Samson et al., 1996), could function as such a cofactor; expression of CKR-5 with huCD4 in otherwise nonpermissive quail and murine cells rendered these cells permissive for syncytia formation mediated by NSI, but not SI, virus env proteins as well as for infection by an M-tropic NSI virus. Furthermore, we found that a dual-tropic primary virus isolate, 89.6, could efficiently utilize both Fusin and CKR-5 as entry cofactors. In addition, the β-chemokine receptors CKR-3 and CKR-2b supported syncytia formation mediated by the 89.6 env protein, indicating that at least some primary virus isolates may utilize an impressive variety of related entry cofactors. The identification of CKR-5 as an entry cofactor for M-tropic HIV strains has important implications for understanding the molecular basis of viral tropism and HIV entry and suggests new possibilities for antiviral strategies. Our finding that HIV-1 89.6 can use both Fusin and CKR-5 as entry cofactors suggests that the evolution from a purely M-tropic NSI phenotype to a T-tropic SI phenotype may result from changes in HIV-1 env that first enable it to use both Fusin and CKR-5 as cofactors.

Results

The β-Chemokine Receptor CKR-5 Functions as a Cofactor for M-Tropic HIV-1 Strains

We have shown that expression of huCD4 in quail QT6 cells, like most other nonhuman cell lines, fails to render them permissive for HIV-1 infection and env-mediated syncytia formation. However, syncytia formation mediated by T cell–tropic (but not M-tropic) env proteins readily occurs when Fusin and huCD4 are coexpressed in QT6 cells (Berson et al., 1996). Expression of Fusin and huCD4 also makes otherwise nonpermissive feline, murine, human, mink, and simian cells suitable targets for T-tropic env-mediated syncytia formation (Berson et al., 1996; Feng et al., 1996). The QT6 cells were chosen for the current study because they can be efficiently transfected.

The similarity between Fusin and the chemokine receptors, coupled with the observations that the β-chemokines RANTES, MIP-1α, and MIP-1β inhibit replication of M-tropic HIV-1 strains in CD4+ T-cells (Cocchi et al., 1995; Paxton et al., 1996), prompted us to employ a syncytia assay to determine if the β-chemokine receptor CKR-5, which binds RANTES, MIP-1α, and MIP-1β (Samson et al., 1996), could function as a cofactor for M-tropic HIV-1 isolates. To do this, huCD4 and either Fusin or CKR-5 were transiently expressed in QT6 cells. After 24 hr, the target QT6 cells were mixed with HeLa cells that had been infected with recombinant vaccinia virus vectors that expressed the env proteins of the T-tropic strain BH8 or the M-tropic strains ADA or JR-FL. The cells were fixed, stained with methylene blue, and examined for syncytia formation 8 hr after mixing. As shown in Figure 1, HeLa cells expressing the T cell–tropic BH8 env protein readily formed syncytia with QT6 cells expressing huCD4 and Fusin but not with cells expressing huCD4 and CKR-5. In contrast, HeLa cells expressing M-tropic env proteins derived from JR-FL or ADA did not fuse with QT6 cells expressing huCD4 and Fusin (JR-FL) or fused very inefficiently (ADA). Only small isolated syncytia were observed with the ADA env protein (Figure 1). However, syncytia were readily observed with M-tropic env proteins when huCD4 and CKR-5 were coexpressed. Fusion was not observed in the absence of CD4 (data not shown) or when a noncleaved fusion-inactive form of env was used (Figure 1). Identical results were obtained when murine PA317 fibroblasts were used as targets (data not shown), indicating that the ability of CKR-5 to support M-tropic env-mediated syncytia formation was not cell type–dependent.

To examine syncytia formation more rigorously, we utilized a content-mixing assay (Broder and Berger, 1995; Nussbaum et al., 1994). In this assay, the target QT6 cells were transfected with plasmids encoding CD4 and either Fusin or CKR-5. In addition, the cells were infected with vCB21r, a recombinant vaccinia virus that expresses β-galactosidase under control of the T7 promoter. HeLa effector cells were infected with recombinant vaccinia viruses that expressed the desired env protein and with vTF1.1, a recombinant vaccinia virus that expresses the T7 polymerase. Target and effector cells were infected overnight and then mixed together and incubated at 37°C for 8 hr. If fusion occurs, the cytoplasmic contents of the target and effector cells mix, leading to β-galactosidase expression (Nussbaum et al., 1994). Fusion can therefore be monitored morphologically by scoring for in situ β-galactosidase activity and biochemically by lysing the cells and measuring β-galactosidase activity, using a quantitative colorimetric assay (Broder and Berger, 1995; Nussbaum et al., 1994).

As shown in Figure 2 and quantitated in Figure 3, HeLa cells expressing HIV-1 BH8 env protein formed syncytia with QT6 cells transfected with plasmids encoding huCD4 and Fusin, but not with cells expressing huCD4 and CKR-5. In contrast, HeLa cells expressing the M-tropic JR-FL env protein formed syncytia with QT6 cells expressing huCD4 and CKR-5 but not with cells expressing huCD4 and Fusin. Fusion was not observed when QT6 cells expressed huCD4 alone (data not shown) or with a noncleaved fusion-inactive form of env (see Figures 2 and 3). Thus, the β-chemokine receptor CKR-5 functions as a cofactor for M-tropic env-mediated membrane fusion.

CKR-5 Supports M-Tropic Virus Infection

To determine if expression of CKR-5 and huCD4 could render QT6 cells permissive for virus infection as well as fusion, CD4 and either Fusin or CKR-5 were transiently expressed in QT6 cells. After 24 hr, cells were infected with the T-tropic HIV-1 strain IIIB or the M-tropic strain JR-FL. The virus inoculum was removed and the cells harvested 24 hr later. To detect virus entry, we used a polymerase chain reaction (PCR)–based entry assay to

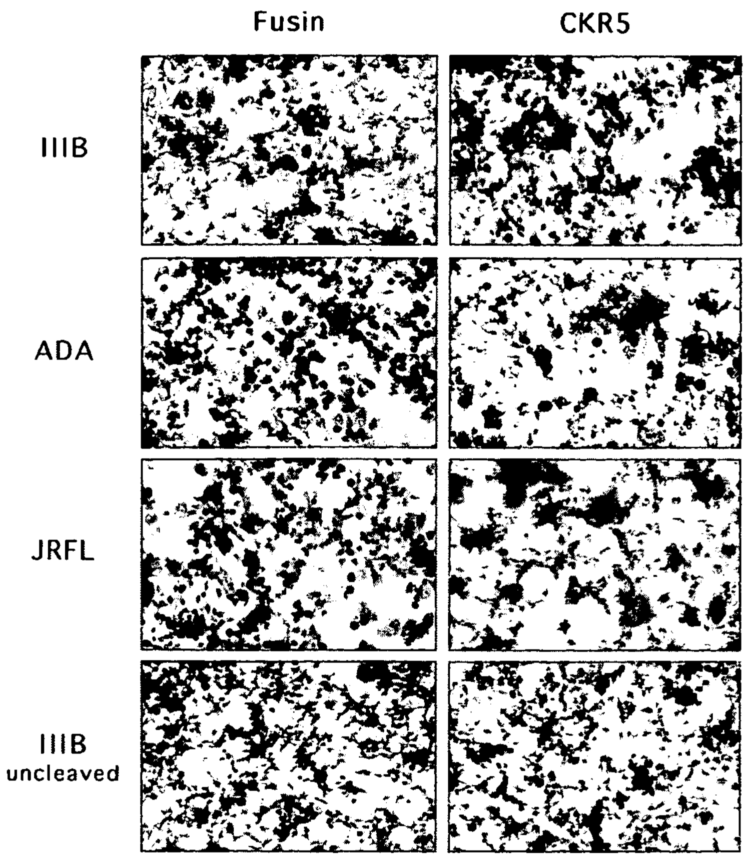


Figure 1. Syncytia Formation Detected by Methylene Blue Staining

QT6 target cells were infected with vCB3 (moi, 10) expressing CD4 and vCB21r (moi, 10) expressing the lacZ gene under the control of the T7 promoter. Within 2–4 hr postinfection, target cells were transfected with pSC59–Fusin expressing Fusin from the vaccinia synthetic early/late promoter, pCDNA3–CKR5 expressing CKR5 from the constitutive CMV promoter, or pCDNA3 vector. HeLa effector cells were infected with vaccinia (moi, 10) expressing the HIV-1 envelope proteins BH8, ADA, JR-FL, or a cleavage-defective form of the BH8 envelope. Effector cells were also infected with vaccinia vTF1.1 (moi, 10) expressing T7-polymerase under the control of the vaccinia late promoter. Cells were allowed to fuse for 8 hr before fixing with glutaraldehyde/formaldehyde and staining with methylene blue.

detect early viral DNA transcripts (Berson et al., 1996). As shown in Figure 4, HIV-1 IIIB entered QT6 cells that expressed both huCD4 and Fusin, consistent with our previous results (Berson et al., 1996). Entry was not observed when CKR-5 was expressed in place of Fusin. In contrast, HIV-1 JR-FL entered QT6 cells expressing huCD4 and CKR-5 but not cells expressing huCD4 and Fusin. Therefore, CKR-5 supported entry of an M-tropic

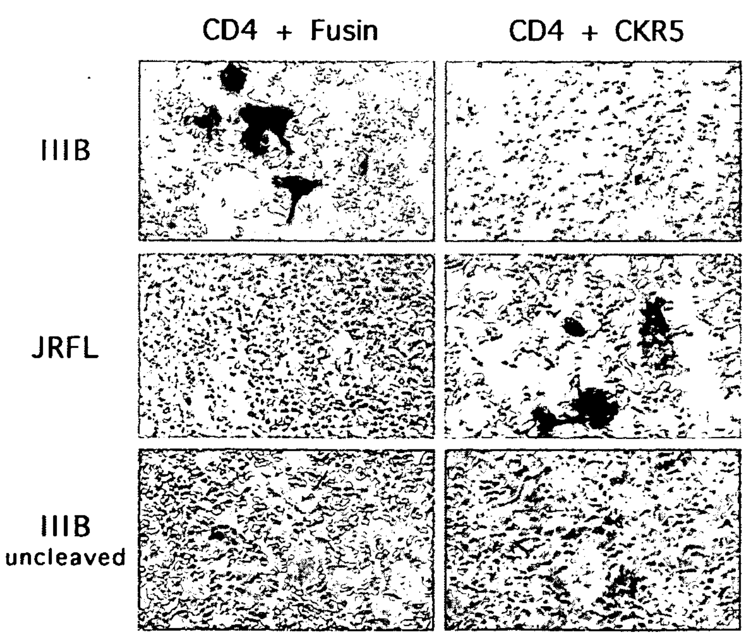


Figure 2. In Situ Detection of Syncytia Formation by Cytoplasmic Mixing and β-Galactosidase Expression

Fusion assays were performed using the same procedure as described in Figure 1, except that pREP8–Fusin expressing Fusin from the Rous sarcoma virus promoter was used in place of pSC59–Fusin. Cells were allowed to fuse for 8 hr before fixing in glutaraldehyde/paraformaldehyde and in situ staining with β-galactosidase substrate, as described in Experimental Procedures.

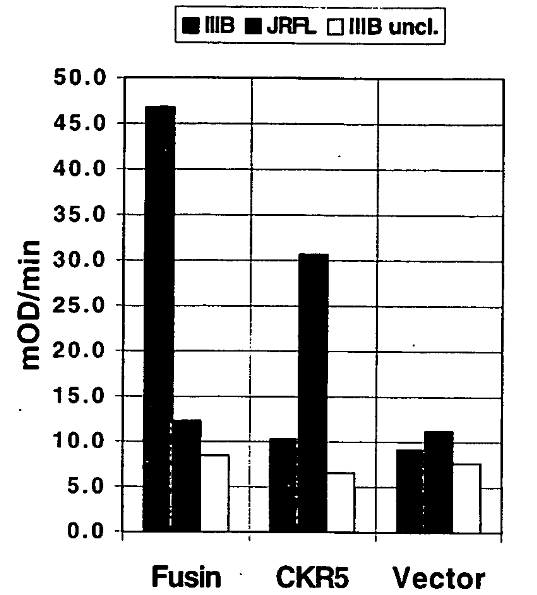


Figure 3. Quantitation of Fusion by β-Galactosidase Activity Assay
The fusion assay was performed using the same procedure as described in Figure 1. Cells were allowed to fuse for 8 hr before lysis in 0.5% NP-40. Cell lysates were harvested and assayed for β-galactosidase activity, as described in Experimental Procedures.

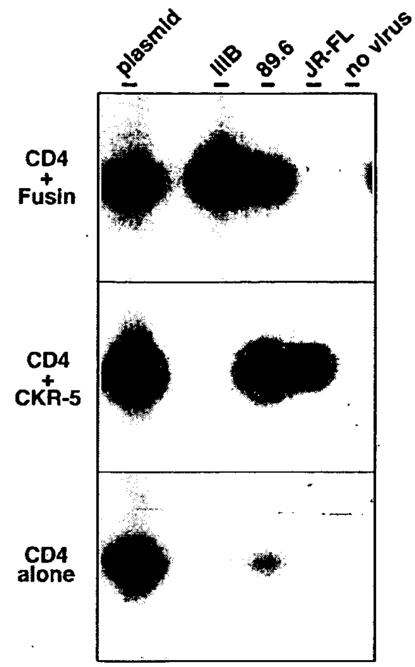


Figure 4. HIV-1 Infection of CD4-Expressing QT6 Target Cells
QT6 cells were cotransfected with pT4 and pSC59–Fusin, pCDNA3–CKR5, or pCDNA3 and then infected with HIV-1 strains BH8, 89.6, JR-FL, or mock supernatant. The following day, cells were lysed and HIV reverse-transcription products detected by PCR amplification of U3/U5 sequences, followed by Southern blot. HIV-1 plasmid served as a positive control. Quail-specific primers were used to demonstrate that equivalent amounts of DNA were used in each sample (data not shown). The small signal seen with 89.6 infection of QT6 cells expressing huCD4 alone was not reproducible and so most likely represents residual virus inoculum; other experiments showed no evidence for virus entry under these conditions.

but not a T-tropic virus strain, consistent with its ability to support membrane fusion mediated by M-tropic env proteins.

A Dual-Tropic HIV-1 Strain Utilizes Both Fusin and CKR-5

Most viruses isolated from asymptomatic individuals replicate in both macrophages and lymphocytes but do not induce syncytia formation in peripheral blood lymphocytes or in transformed cell lines (Conner and Ho, 1994; Conner et al., 1993; Schuitemaker et al., 1991, 1992). With disease progression, more cytopathic T-tropic SI viruses emerge. Whether cytopathic variants evolve from M-tropic NSI viruses or emerge from strains that are suppressed but present during the early stages of infection is not known. However, viruses with intermediate phenotypes have been described, supporting the idea that cytopathic viruses may evolve from noncytopathic strains via a transitional dual-tropic phenotype. We examined one such dual-tropic virus, 89.6, a primary virus isolate that retains the ability to replicate efficiently in macrophages in spite of having SI characteristics and the ability to replicate in some transformed T cell lines (Collman et al., 1992). To determine if the properties of 89.6 could be explained by the ability of 89.6 env to utilize multiple-entry cofactors, we tested the ability of the 89.6 env protein to fuse with QT6 cells expressing huCD4 and either Fusin or CKR-5. We found that HeLa cells expressing the env protein derived from 89.6 failed to form syncytia with QT6 cells that expressed huCD4 alone, indicating that this dual-tropic virus required one or more entry cofactors. In contrast, HeLa cells expressing 89.6 env readily formed syncytia with QT6 cells expressing huCD4 and either Fusin or CKR-5 (see Figure 5). Syncytia formation did not occur in the absence of CD4.

To obtain quantitative fusion data, we again used a gene reporter assay. Because the recombinant vaccinia virus that expresses the 89.6 env protein also constitutively expresses β-galactosidase, target QT6 cells were transfected with a plasmid containing luciferase, rather than lacZ, under control of the T7 promoter. Using this sensitive assay, we found that HeLa cells expressing the 89.6 env protein readily fused to QT6 target cells expressing huCD4 and Fusin and also to QT6 cells expressing huCD4 and CKR-5 (see Figure 6A). By contrast, HeLa cells expressing the T-tropic BH8 env protein formed syncytia only with cells expressing Fusin, while those expressing the M-tropic JR-FL env protein only fused with cells expressing CKR-5. Finally, we performed the PCR–entry assay to determine if both Fusin and CKR-5 could support virus entry into QT6 cells in conjunction with huCD4. As shown in Figure 4, we found that HIV-1 89.6 virus could use both Fusin and CKR-5 as entry cofactors, consistent with the membrane fusion results shown in Figures 5 and 6A. Thus, the dual-tropic nature of HIV-1 89.6 can be explained by its ability to use two distinct entry cofactors. A small signal was obtained when QT6 cells expressing huCD4 alone were infected with 89.6 (Figure 4), but this result was not

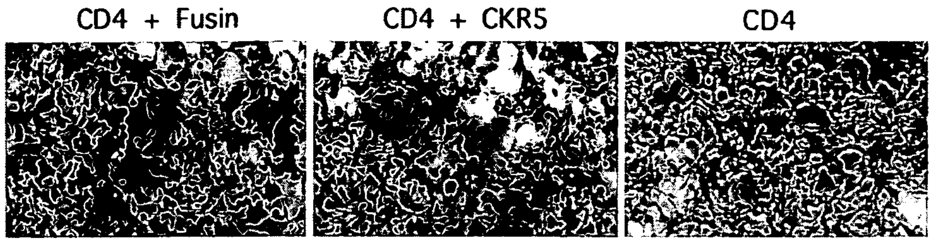


Figure 5. Syncytia Formation by 89.6 env Detected by Methylene Blue Staining

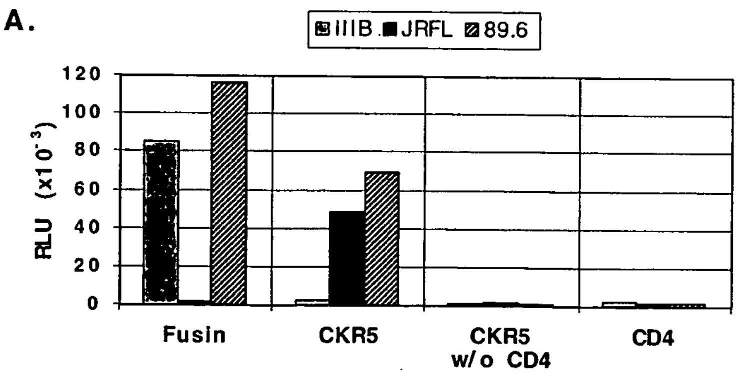
QT6 target cells were transfected with pREP8–Fusin expressing Fusin from the constitutive Rous sarcoma virus promoter, pCDNA3–CKR5 expressing CKR5 from the constitutive CMV promoter, or pCDNA3 vector. All target cells were also transfected with pT4, which constitutively expresses CD4 from the CMV promoter. HeLa effector cells were infected with vBD3 (moi, 10) expressing the 89.6 envelope. Cells were allowed to fuse for 8–10 hr before fixing with methanol and staining with methylene blue.

reproducible. Other experiments demonstrated no evidence of 89.6 entry into QT6 cells expressing huCD4 alone (consistent with the syncytia formation assay results in Figures 3 and Figure 6A), suggesting that this small signal was due to residual virus inoculum.

CKR-3 and CKR-2b Support 89.6 env-Mediated Membrane Fusion

The ability of HIV-1 89.6 to use both Fusin and CKR-5 as entry cofactors despite their divergent sequences (approximately 21% amino acid homology in the extracellular domains) prompted us to examine whether other chemokine receptors could function as cofactors for this dual-tropic virus. QT6 cells were transfected with plasmids encoding huCD4 as well as the β-chemokine receptors CKR-3 (which is expressed in eosinophils) and CKR-2b (which is expressed in monocytes). In addition, we transfected QT6 cells with a plasmid encoding the Duffy blood group antigen, a seven-transmembrane receptor that bears approximately 20% homology to Fusin and has been shown to bind to RANTES, IL-8, and MCP-1 (Chaudhuri et al., 1994; Neote et al., 1994; Peiper et al., 1995). Work by Dragic et al. (1995), demonstrating that fusion of human red blood cell ghosts with murine cells expressing huCD4 makes these cells susceptible for syncytia formation mediated by a T-tropic HIV-1 env protein, suggested that the Duffy antigen might serve as a HIV-1 cofactor. In the presence of huCD4, we found that both CKR-3 and CKR-2b supported syncytia formation by the 89.6 env protein (Figure 6B), but not by the BH8 and JR-FL env proteins. The Duffy blood group antigen failed to support membrane fusion by any of

A.



B.

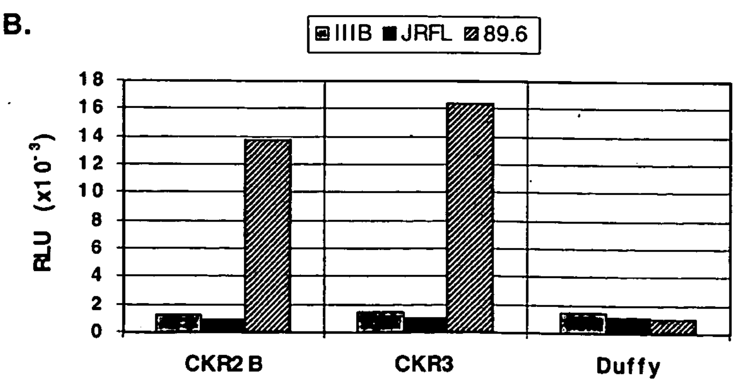


Figure 6. Quantitation of 89.6 Fusion by Luciferase Assay

(A) QT6 target cells were transfected with pREP8–Fusin expressing Fusin from the constitutive Rous sarcoma virus promoter, pCDNA3–CKR5 expressing CKR5 from the constitutive CMV promoter, or pCDNA3 vector. All target cells, except where indicated, were transfected with pT4, which constitutively expresses CD4 from the CMV promoter, and cells that did not receive CD4 were transfected with pCDNA3 vector. All target cells were also transfected with a plasmid containing the luciferase gene driven by a T7 promoter (Promega). HeLa effector cells were infected with vBD3 (moi, 10), which expresses the 89.6 envelope protein, or with viruses that express the JR-FL or BH8 env proteins. All effector cells were infected with vTF1.1 (moi, 10), which expresses T7 polymerase under the control of the vaccinia late promoter. Cells were allowed to fuse for 6 hr before lysis in Reporter Lysis Buffer (Promega) and assay for luciferase activity. Results are expressed in terms of relative light units (RLU), and raw data has been multiplied by 10^{-3}.

(B) This experiment was performed in an identical fashion to that shown in (A), except that the target cells were transfected with plasmids expressing CKR-3, CKR-2b, or the Duffy blood group antigen.

the env proteins tested. While the efficiency of fusion mediated by the different cofactors appeared to vary, the relative efficiency of Fusin, CKR-5, CKR-3, and CKR-2b to support syncytia formation must await development of specific antibodies that will allow quantitation of surface expression levels of these membrane fusion cofactors.

Discussion

HIV-1 isolates can be placed into three groups based on their ability to infect distinct target cell populations. Viruses that are M-tropic and that fail to form syncytia in culture represent the most prevalent phenotype isolated from individuals shortly after seroconversion and during the asymptomatic period of the disease (Conner and Ho, 1994; Conner et al., 1993; Roos et al., 1992; Schuitemaker et al., 1991, 1992; Tersmette et al., 1988, 1989b; Zho et al., 1993). By contrast, viruses that exhibit a T-tropic SI phenotype typically emerge later in infection and are associated with CD4+ T cell decline and progression to AIDS (Tersmette et al., 1988, 1989a, 1989b). Dual-tropic viruses retain the ability to replicate efficiently in macrophages despite exhibiting SI characteristics and may represent an important transitional phenotype in the evolution of T-tropic SI viruses from the M-tropic NSI viruses present early in the course of the disease (Collman et al., 1992; Shibata et al., 1995). An important factor governing cell tropism is the ability of a virus to enter different target cells. In the case of HIV-1, T-tropic viruses generally fail to enter macrophages, while M-tropic viruses often fail to enter transformed T cell lines in spite of the fact that both target cells express the primary viral receptor, CD4. Thus, CD4 by itself is not sufficient for virus entry. In addition, expression of huCD4 in nonhuman (and some human) cell lines typically fails to render them permissive for virus infection and env-mediated syncytia formation, supporting the idea that a cofactor in addition to CD4 is required for HIV-1 entry (Ashorn et al., 1990; Broder and Berger, 1995; Broder et al., 1993; Chesebro et al., 1990; Clapham et al., 1991; Dragic et al., 1992, 1995; Harrington and Geballe, 1993; Maddon et al., 1986). Only recently has a bona fide HIV-1 specific cofactor been identified, an orphan seven-transmembrane domain receptor termed Fusin (Berson et al., 1996; Feng et al., 1996).

Fusin is most closely related to the α- and β-chemokine receptor families (Federsppiel et al., 1993; Herzog et al., 1993; Jazin et al., 1993; Loetscher et al., 1994; Nomura et al., 1993). However, functional interactions between Fusin and various chemokines have not been detected (Herzog et al., 1993; Jazin et al., 1993; Loetscher et al., 1994; Nomura et al., 1993). Fusin exhibits a broad tissue distribution and is expressed at high levels in B-, T-, and monocyte-derived cell lines (Federsppiel et al., 1993; Herzog et al., 1993; Jazin et al., 1993; Loetscher et al., 1994; Nomura et al., 1993). Expression of Fusin and huCD4 in otherwise nonpermissive cell lines renders them susceptible to T-tropic env-mediated syncytia formation as well as infection by T-tropic SI viruses (Berson et al., 1996; Feng et al., 1996). However, Fusin either is not used by M-tropic NSI viruses as a cofactor or, as appears to be the case of the M-tropic strain ADA, is used very inefficiently (Berson et al., 1996; Feng et al., 1996). Given the high degree of homology between M- and T-tropic env proteins, we speculated that cofactors used by M-tropic viruses would be structurally related to Fusin. The similarity between Fusin and the chemokine receptor families, coupled with recent work showing that the β-chemokines RANTES, MIP-1α, and MIP-1β have significant antiviral activity against M-tropic but not T-tropic virus strains (Cocchi et al., 1995; Paxton et al., 1996), made the β-chemokine receptor CKR-5 a likely candidate.

We found that expression of CKR-5 in conjunction with huCD4 in otherwise nonpermissive quail QT6 or murine PA317 cells made these cells suitable targets for M-tropic env-mediated syncytia formation and also allowed virus entry. It is important to note that CKR-5 by itself did not support membrane fusion: fusion was only observed when target cells expressed both huCD4 and CKR-5. Expression of huCD4 and CKR-5 did not support fusion or virus entry by the T-tropic strain IIIB. CKR-5 contains 352 amino acids and has a predicted molecular mass of 40,600 kDa (Samson et al., 1996). It exhibits between 49% and 76% amino acid homology with other β-chemokine receptors, being most closely related to CKR-2b. Structural motifs include a single potential N-linked glycosylation site present in the third extracellular loop and four conserved cysteine residues in the ectodomain, with disulfide bonds predicted to occur between cysteines in the first and second extracellular loops and between the cysteine pair in the N-terminal domain and third extracellular loop (Samson et al., 1996; Strader et al., 1994). CKR-5 contains a serine/threonine-rich cytoplasmic domain that is a likely target for phosphorylation and contains a number of conserved proline residues in its transmembrane domains. Functionally, addition of RANTES, MIP-1α, and MIP-1β to cells expressing CKR-5 induces functional responses, though direct chemokine binding has not yet been demonstrated (Samson et al., 1996). CKR-5 shares approximately 30% amino acid homology with Fusin, with 38% homology between the transmembrane domains, 30% between the intracellular loops, and 21% between the extracellular domains. The first extracellular loop is the most highly conserved ectodomain region, with 7 of 14 residues being identical between Fusin and CKR-5.

Viruses representing evolutionary transitions between T- and M-tropic strains may be unique tools for linking disease manifestation with the underlying cellular basis of viral phenotype. The identification of Fusin as a cofactor for T-tropic viruses and CKR-5 as a cofactor for M-tropic viruses enabled us to determine if the dual-tropic nature of 89.6 could be explained by the use of both cofactors. HIV-1 89.6, isolated from peripheral blood mononuclear cells, replicates well in macrophages and in peripheral blood lymphocytes (Collman et al., 1992). However, unlike most M-tropic viruses, infection of peripheral blood lymphocytes with 89.6 results in syncytia formation. In addition, 89.6 can productively infect some transformed cell lines, such as CEMX174. We found that despite their divergent sequences, both Fusin and CKR-5 supported membrane

fusion mediated by the 89.6 env protein, and both supported 89.6 virus entry. The 89.6 env protein has 88%–91% amino acid homology with well-characterized M- and T-tropic viruses but does not exhibit greater homology to either group (Collman et al., 1992). The V3 loop of 89.6, a region of gp120 that has been shown to influence cell tropism, reflects this; it diverges almost equally from T- and M-tropic viruses. The ability of HIV-1 89.6 to utilize both CKR-5 and Fusin suggests that the cofactors share conserved conformational features or general structural characteristics, such as charge, that may be important for cofactor function. The ability of CKR-3 and CKR-2b to be used as fusion cofactors supports this idea and suggests that any env–cofactor interactions will be complex in nature. Alternatively, env proteins may harbor distinct sites that govern cofactor usage, and 89.6 may possess several such sites. Regardless, the ability of 89.6 to use both Fusin and CKR-5 suggests that relatively subtle changes in the env protein can result in the ability to use both entry cofactors. Such dual tropism could play an important role in the transition from M-tropic NSI to T-tropic SI HIV-1 strains during the course of disease. It will be interesting to determine if the high levels of RANTES, MIP-1α, and MIP-1β secreted by CD4+ lymphocytes from some HIV-1 infected individuals provide the selective pressure to drive the evolution of cofactor usage (Paxton et al., 1996).

The ability of 89.6 to utilize molecules as divergent as Fusin and CKR-5 as entry cofactors prompted us to determine if molecules more closely related to CKR-5 could likewise be used by this virus. The β-chemokine receptors CKR-3 (58% homology to CKR-5) and CKR-2b (76% homology to CKR-5) functioned as fusion cofactors for 89.6 env, while the Duffy blood group antigen (19% homology) did not. Expression of CKR-3 appears to be restricted to eosinophils (Combadiere et al., 1995b; Kitaura et al., 1996), making it unlikely that it could serve as an HIV-1 cofactor in vivo. However, more detailed studies on the distribution of CKR-3, as well as other chemokine receptors, will be needed. CKR-2b, however, is expressed in monocytes (Combadiere et al., 1995a) and so may be a biologically relevant cofactor for some HIV-1 strains.

The finding that fusion of either protease or heat-treated human red blood cell ghosts with murine cells expressing huCD4 rendered these cells competent for HIV-1 env-mediated membrane fusion indicated that red cell membranes contain one or more entry cofactors (Dragic et al., 1992; Puri et al., 1996). We do not yet know if human red cells express any of the known HIV cofactors, but the presence of the Duffy blood-group antigen, a promiscuous chemokine receptor, is intriguing. However, we found that coexpression of Duffy and huCD4 in quail cells failed to support syncytia formation by three different HIV-1 env proteins. While it is possible that red cells may contain an as yet unidentified cofactor, it is also possible that processing differences could account for these observations. The ability of Duffy to serve as a HIV-1 cofactor could also be tested by repeating the experiments of Dragic et al. (1992), using blood cells from individuals that lack the Duffy antigen.

Fusin and CKR-5 are likely to play a role in enabling the membrane-fusion reaction that is required for viral penetration, since expression of huCD4 alone in a nonpermissive cell enables virus to bind but not to fuse with the target cell. A series of discrete steps must occur in order for HIV-1 to infect a cell. First, the virus must bind to the surface of the target cell, which generally involves a high affinity interaction between env and CD4. Next, a triggering event must elicit a conformational change in env that leads to membrane coalescence and fusion. Only after membrane fusion can the viral capsid enter the host cell cytoplasm. Therefore, the env protein plays two critical roles in viral entry: receptor binding and membrane fusion. While CD4 by itself is sufficient for binding env, it is not sufficient to trigger the entire series of conformational changes in env that are required for fusion to occur. Fusin and CKR-5 may provide the trigger that allows fusion to occur. A possible scenario is that conformational changes in env that result from CD4 binding could, in turn, allow env to interact with Fusin or CKR-5, leading to additional conformational changes that result in exposure of the fusion peptide and membrane fusion. It will be important to test this hypothesis and to determine if env interacts directly with the viral cofactors in either a CD4-dependent or -independent manner. The cofactors may also interact directly with CD4 and perhaps alter its conformation or presentation on the cell surface.

The identification of Fusin and CKR5 as entry cofactors has important implications for understanding viral entry, tropism, and pathogenesis, as well as the generation of transgenic animals that could serve as models for HIV infection. It also opens new areas for potential therapeutic strategies. The β-chemokines RANTES, MIP-1α, and MIP1-β have significant antiviral activity against M-tropic, but not T-tropic, HIV-1 strains (Cocchi et al., 1995; Paxton et al., 1996). The mechanism by which these chemokines exert their antiviral activity may be through receptor blockade or down-regulation. These or other compounds that prevent cofactor usage may be effective antiviral agents. It will also be important to determine if other proteins can serve as viral cofactors. Given the impressive genetic diversity exhibited by HIV-1 strains and the fact that 89.6 can use at least four distinct molecules as fusion cofactors, it will not be surprising if additional molecules may be able to function as cofactors for different HIV-1, HIV-2, and simian immunodeficiency virus strains. CKR-5, for example, is more closely related to other β-chemokine receptors than to Fusin, including CKR-1 (56% homology) which also binds RANTES, MIP-1α, and MIP-1β (Neote et al., 1993), and CKR-4 (49% homology) which binds to RANTES, MIP-1α, and MCP-1 (Power et al., 1995). The use of primary virus strains as well as viruses from different clades may reveal additional complexities. Finally, the ability of 89.6 to use both Fusin and CKR-5 as entry cofactors provides a molecular explanation for its dual-tropic phenotype and provides evidence that evolution of T-tropic SI viruses from M-tropic NSI viruses may involve a transitional phenotype that exhibits a broader range of cofactor usage.

Experimental Procedures

Constructs

pSC59–Fusin and pREP8–Fusin constructs have been previously described (Berson et al., 1996). CKR-5 was cloned into pCDNA3

(Invitrogen), using the upstream BamHI and downstream XBAI sites for expression using the cytomegalovirus (CMV) promoter. The luciferase–T7 plasmid was obtained from Promega. The plasmid pT4, providing expression of huCD4 under the control of the CMV promoter, was provided by Dr. Dennis Kolson (University of Pennsylvania). pTLZ, encoding lacZ under the control of the T7 promoter, was provided by Dr. Chris Broder (National Institute of Allergy and Infectious Diseases, National Institutes of Health). The 89.6 envelope gene was cloned into the vaccinia virus expression vector pSC59 by PCR. Internal regions of the gene were replaced with sequences not subject to PCR by restriction-fragment swaps, and all areas subject to PCR were confirmed by sequence analysis. The 5′ end of the clone was defined with PCR primer TCATTCGTCGACAGATTA ATTGATA, which introduces a SalI restriction site 46 bp before the 89.6 env start codon. The 3′ end of the clone utilizes the natural BspEI site approximately 600 bp after the 89.6 env natural stop codon. The Sal-to-BspEI sequences were inserted into pSC59 using the 5′ SalI and 3′ Xma sites in pSC59 and a BspEI–Xma linker sequence previously introduced into the vector.

Cells

The human cervical carcinoma cell line HeLa was obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, NIH). The murine embryo fibroblast cell line PA317T4 (McKnight et al., 1994) was a gift from Dr. James Simon (University of Pennsylvania). The Japanese quail fibrosarcoma cell line QT6-C5 (QT6; ATCC# CRL-1708) was provided by Paul Bates (University of Pennsylvania). HuTK− 143B fibroblasts (#CRL-8303) were obtained from the American Type Culture Collection.

Tissue culture media and supplements were purchased from Life Technologies, Inc. unless otherwise noted. HeLa, PA317T4, and HuTK− were maintained in Dulbecco's modified Eagle's media: high glucose, supplemented with 10% fetal bovine serum (Hyclone). QT6 cells were maintained in Medium 199, supplemented with 10% tryptose phosphate broth (Sigma), 5% fetal bovine serum, and 1% chicken serum. All media were supplemented with 2 mM glutamine and penicillin–streptomycin.

Viruses

Dr. Christopher Broder of the NIAID kindly provided us with the following panel of recombinant vaccinia viruses encoding the envs of several HIV-1 strains (indicated in parentheses; Broder and Berger, 1995): vSC60 (IIIB, BH8 clone), vCB39 (ADA), and vCB28 (JR-FL). The recombinant vaccinia virus vCB16, encoding a nonfusogenic uncleaved BH8 env was used as a negative control. Dr. Broder also provided us with the recombinant viruses vCB3 (Broder and Berger, 1995), encoding huCD4; vTF1.1, encoding the T7 RNA polymerase; and vCB21r, encoding lacZ under control of the T7 promoter. The recombinant vaccinia virus vBD3, expressing the 89.6 full-length envelope protein under control of the vaccinia virus early/late promoter was generated using standard techniques utilizing thymidine kinase–negative selection (Earl, 1991). Vaccinia virus was created by transfecting the pSC59/89.6 gp160 plasmid into CV-1 cells infected with the wild-type vaccinia virus strain WR. Recombinants were screened by expression of lacZ and were plaque-purified five times to homogeneity.

Gene Reporter Fusion Assay

To quantitate cell–cell fusion events, we utilized a modified version of the gene reporter fusion assay described by Nussbaum et al. (1994). T7 RNA polymerase and env proteins were introduced into effector HeLa cells by infection with recombinant vaccinia viruses. Target QT6 cells were transfected with CD4, Fusin, or CKR5, as indicated in the text and legends. For quantitative assays, a reporter gene coding for either β-galactosidase or luciferase under the control of the T7 promoter was transfected into the target cells. Vaccinia-encoded proteins were produced by infecting cells at a multiplicity of infection (moi) of 10 for 1.5–4 hr at 37°C. Effector cells were then trypsinized, washed with phosphate-buffered saline, resuspended in media, and incubated at 32°C overnight in the presence of rifampicin. Proteins were generally introduced into target QT6 cells in 24 well plates by transfection of 2 μg of each plasmid, using the calcium-phosphate precipitation method. The transfectant was removed after 4 hr, and the cells were incubated at 37°C overnight. In some experiments, as indicated in the figure legends, CD4 and T7–β-galactosidase were introduced by infection with recombinant vaccinia viruses at a moi of 10. Cells were infected 2–3 hr prior to transfection. In infection/transfection experiments, overnight incubation was at 32°C in the presence of rifampicin. Transfection and infection/transfection of target cells yielded identical results, although infection experiments generally caused cytopathic effects and increased background.

To initiate fusion, target and effector cells were mixed in 24 well plates at 37°C in the presence of ara-C and rifampicin and allowed to fuse for 8–10 hr. Syncytia formation was monitored by fixing the cultures in 0.29% glutaraldehyde/1% formaldehyde/phosphate-buffered saline and staining with 0.5% methylene blue, 0.17% pararosaniline in methanol. To quantitate fusion, NP-40 was added to a final concentration of 0.5%, and aliquots of the cell lysates were monitored for β-galactosidase activity using the colorimetric assay described by Nussbaum et al. (1994). In some cases, β-galactosidase was detected by in situ staining. Since the 89.6 env-encoding vaccinia virus expressed the lacZ gene, quantitation of 89.6 env-mediated fusion was measured using a luciferase assay. After 8–10 hr of fusion, 2–3 × 10^5 cells were lysed in 150 μl of reporter lysis buffer (Promega) and assayed for luciferase activity according to the instructions of the manufacturer (Promega).

Infection Studies

pREP8–Fusin or pcDNA3–CKR5 were cotransfected with pT4 (2 μg of each plasmid) into 2 × 10^5 QT6 cells in 24 well tissue culture plates, using calcium-phosphate precipitation. The next day, cells were infected with DNase-treated (15 U/ml for 30 min at room temperature) cell-free virus stock for 4 hr at 37°C, using 75 ng p24 antigen each of strains JR-FL, 89.6, or IIIB (derived from clone HXB2). Following infection, cells were washed and fed. The cells were detached with trypsin 24 hr later, pelleted, suspended in 50 μl lysis buffer (100 mM KCl, 20 mM Tris [pH 8.4], 0.1% NP-40, 500 μg/ml proteinase K), and incubated for 2 hr at 60°C, followed by boiling for 15 min.

HIV detection by PCR was performed for 35 cycles on 2.5 μl of cell lysate to amplify a 430 bp region of U3/U5 LTR DNA sequences, using primers LTR-plus/LTR-minus (5′-ACAAGCTAGTACCAGTTGA GCC-3′; 5′-CACACACTACTTGAAGCACTCA-3′). Products were resolved by electrophoresis on 2% agarose gels, transferred to Hybond N+ (Amersham) and probed with a [^{32}P] end-labeled oligonucleotide probe (5′-ATCTACAAGGGACTTTCCCGC-3′) followed by autoradiography. To ensure that equivalent amounts of total cellular DNA were amplified, quail cell–specific primers Q101-plus/Q261-minus (5-GGTAACGGTTCTTTGTCCCGTTGC-3′; 5′-CGC GCTGGTCCCGCAGCCCCACTC-3′) were used to amplify a 153 bp region of the avian sarcoma virus receptor tvA, which is present in QT6 cell genomic DNA (Bates et al., 1993), which was visualized by ethidium bromide staining.

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 NEWS  8  MAY 30  The F-Term thesaurus is now available in CA/CAplus
 NEWS  9  JUN 02  The first reclassification of IPC codes now complete in
                  INPADOC
 NEWS 10  JUN 26  TULSA/TULSA2 reloaded and enhanced with new search and
                  and display fields
 NEWS 11  JUN 28  Price changes in full-text patent databases EPFULL and PCTFULL
 NEWS 12  JUl 11  CHEMSAFE reloaded and enhanced
 NEWS 13  JUl 14  FSTA enhanced with Japanese patents
 NEWS 14  JUl 19  Coverage of Research Disclosure reinstated in DWPI
 NEWS 15  AUG 09  INSPEC enhanced with 1898-1968 archive
 NEWS 16  AUG 28  ADISCTI Reloaded and Enhanced
 NEWS 17  AUG 30  CA(SM)/CAplus(SM) Austrian patent law changes
 NEWS 18  SEP 11  CA/CAplus enhanced with more pre-1907 records
 NEWS 19  SEP 21  CA/CAplus fields enhanced with simultaneous left and right
                  truncation
 NEWS 20  SEP 25  CA(SM)/CAplus(SM) display of CA Lexicon enhanced
 NEWS 21  SEP 25  CAS REGISTRY(SM) no longer includes Concord 3D coordinates
 NEWS 22  SEP 25  CAS REGISTRY(SM) updated with amino acid codes for pyrrolysine
 NEWS 23  SEP 28  CEABA-VTB classification code fields reloaded with new
                  classification scheme

 NEWS EXPRESS  JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 JUNE 2006.

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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 12 Oct 2006 (20061012/PD)
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USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Jun 2006


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E1             8      DOMS LUTZ/IN
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E3            12 --> DOMS ROBERT W/IN
E4             1      DOMSA KAROLY/IN
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E9             2      DOMSCHITZ JOCHEN/IN
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E11 11 DOMSCHKE ANGELIKA/IN
E12 5 DOMSCHKE ANGELIKA MARIA/IN

=> s e3
L1 12 "DOMS ROBERT W"/IN

=> d l1,ti,1-12

L1 ANSWER 1 OF 12 USPATFULL on STN
TI CD4-independent HIV envelope proteins as vaccines and therapeutics

L1 ANSWER 2 OF 12 USPATFULL on STN
TI ADMINISTRATION OF AN AGENT WHICH DECREASES PROCESSING OF AMYLOID PRECURSOR PROTEIN

L1 ANSWER 3 OF 12 USPATFULL on STN
TI CD4-independent HIV envelope proteins as vaccines and therapeutics

L1 ANSWER 4 OF 12 USPATFULL on STN
TI Methods and compositions for modulating the interaction between the APJ receptor and the HIV virus

L1 ANSWER 5 OF 12 USPATFULL on STN
TI Methods and compositions for modulating the interaction between the APJ receptor and the HIV virus

L1 ANSWER 6 OF 12 USPATFULL on STN
TI Methods and compositions for modulating the interaction between the APJ receptor and the HIV virus

L1 ANSWER 7 OF 12 USPATFULL on STN
TI Methods and compositions for modulating the interaction between the APJ receptor and the HIV virus

L1 ANSWER 8 OF 12 USPATFULL on STN
TI Lipoparticle comprising a protein and methods of making and using the same

L1 ANSWER 9 OF 12 USPATFULL on STN
TI CD4-independent HIV envelope proteins as vaccines and therapeutics

L1 ANSWER 10 OF 12 USPATFULL on STN
TI METHODS AND COMPOSITIONS FOR MODULATING THE INTERACTION BETWEEN THE APJ RECEPTOR AND THE HIV VIRUS

L1 ANSWER 11 OF 12 USPATFULL on STN
TI Oligomeric HIV-1 envelope glycoproteins

L1 ANSWER 12 OF 12 USPATFULL on STN
TI Oligomeric HIV-1 envelope glycoproteins

=> d l1,cbib,clm,1-12

L1 ANSWER 1 OF 12 USPATFULL on STN
2005:220886 CD4-independent HIV envelope proteins as vaccines and therapeutics.
Hoxie, James A., Berwyn, PA, UNITED STATES
LaBranche, Celia C., Chapel Hill, NC, UNITED STATES
Doms, Robert W., Berwyn, PA, UNITED STATES
Hoffman, Trevor L., Lansdowne, PA, UNITED STATES
US 2005191621 A1 20050901
APPLICATION: US 2005-48554 A1 20050201 (11)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1-18. (canceled)

19. A pharmaceutical composition comprising a CD4-independent HIV-1 Env protein, wherein said HIV-1 Env comprises at least one mutation causing the chemokine coreceptor binding site to be stably exposed.

20. The composition of claim 21, wherein said HIV-1 Env is HIV-1/IIIBx 8x.

21. A vaccine comprising an immunogenic dose of a CD4-independent HIV-1 Env.

22. The vaccine of claim 21, wherein said HIV-1 Env is selected from the group consisting of a HIV-1 Env polypeptide, a nucleic acid encoding HIV-1 Env, and a cell expressing HIV-1 Env.

23-51. (canceled)

L1 ANSWER 2 OF 12 USPATFULL on STN
2003:265300 ADMINISTRATION OF AN AGENT WHICH DECREASES PROCESSING OF AMYLOID PRECURSOR PROTEIN.
LEE, VIRGINIA M.-Y., PHILADELPHIA, PA, UNITED STATES
DOMS, ROBERT W., BERWYN, PA, UNITED STATES
US 2003186315 A1 20031002
APPLICATION: US 1999-297877 A1 19990628 (9)
WO 1997-US20212 19971113
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method of identifying agents which increase or decrease processing of amyloid precursor protein into amyloid β peptides found in neuritic plaques and vascular deposits that accumulate in brains of patients with Alzheimer's disease comprising: contacting NT2N cells with a compound or agent suspected of increasing or decreasing amyloid precursor protein processing; and measuring levels of amyloid P peptides formed in the endoplasmic reticulum of the cells.

2. A method of diagnosing Alzheimer's disease in a patient comprising detecting in the patient an agent identified to increase processing of amyloid precursor protein into amyloid β peptides found in neuritic plaques and vascular deposits that accumulate in brains of patients with Alzheimer's disease in accordance with the method of claim 1.

3. A method of inhibiting processing of amyloid precursor protein into amyloid β peptides found in neuritic plaques and vascular deposits that accumulate in brains of patients with Alzheimer's disease comprising administering to the patient an agent which decreases processing of amyloid precursor protein into amyloid β peptides found in neuritic plaques and vascular deposits that accumulate in brains of patients with Alzheimer's disease wherein said agent is identified in accordance with the method of claim 1.

L1 ANSWER 3 OF 12 USPATFULL on STN
2003:133509 CD4-independent HIV envelope proteins as vaccines and therapeutics.
Hoxie, James A., Berwyn, PA, UNITED STATES
LaBranche, Celia C., Chapel Hill, NC, UNITED STATES
Doms, Robert W., Berwyn, PA, UNITED STATES
Hoffman, Trevor L., Lansdowne, PA, UNITED STATES
University of Pennsylvania (U.S. corporation)
US 2003091594 A1 20030515
APPLICATION: US 2002-196515 A1 20020716 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An isolated nucleic acid encoding a CD4-independent human immunodeficiency virus-1 (HIV-1) env, or a mutant, derivative, or fragment thereof.

2. The isolated nucleic acid of claim 1, wherein said nucleic acid shares at least about 98% homology with the nucleic acid having the nucleotide sequence of SEQ ID NO:4.

3. The isolated nucleic acid of claim 2, wherein said nucleic acid is selected from the group consisting of an HIV-1/IIIBx env, and an HIV-1/IIIBx 8x (8x) env.

4. The isolated nucleic acid of claim 3, wherein said nucleic acid is an HIV-1/IIIBx 8x env.

5. An isolated nucleic acid encoding a CD4-independent HIV env having the nucleotide sequence of SEQ ID NO:4.

6. An isolated nucleic acid comprising a portion of a HIV-1 env gene which confers CD4 independence on at least one HIV-1 env clone.

7. A chimeric nucleic acid comprising a first portion and a second portion, said first portion encoding at least a portion of an HIV-1/IIIBx 8x env coding sequence and said second portion encoding at least a portion of an HIV-1 env coding sequence which is not an 8x env.

8. The chimeric nucleic acid of claim 7, wherein said second portion is an env coding sequence selected from the group consisting of an S10 env, an HXB2 env, a BaL env, and an IIIB env.

9. The chimeric nucleic acid of claim 7, wherein said second portion comprises a chemokine receptor binding site selected from the group consisting of a CXCR4 chemokine receptor binding site, and a CCR5 chemokine receptor binding site.

10. The chimeric nucleic acid of claim 9, wherein said second portion comprises a V3-loop coding sequence selected from the group consisting of a V3-loop for a CXCR4 chemokine receptor binding site, and a V3-loop for a CCR5 chemokine receptor binding site.

11. An isolated HIV-1 gp120 polypeptide comprising a stably exposed chemokine coreceptor binding site.

12. An isolated polypeptide comprising an HIV-1/IIIBx 8x Env.

13. The isolated polypeptide of claim 12, wherein said polypeptide shares at least about 98% homology with SEQ ID NO:3.

14. The isolated polypeptide of claim 13 comprising the amino acid sequence of SEQ ID NO:3.

15. A chimeric HIV-1 Env polypeptide comprising a gp120 polypeptide wherein said chimeric polypeptide comprises a first portion comprising an HIV-1/IIIBx 8x gp120, said chimeric polypeptide further comprising a second portion comprising a gp120 from an HIV-1 other than HIV-1/IIIBx 8x.

16. A chimeric HIV-1 Env polypeptide wherein said polypeptide is CD4-independent, and further wherein said polypeptide comprises a chemokine receptor binding site selected from the group consisting of a CXCR4 chemokine receptor binding site, and a CCR5 chemokine receptor binding site.

17. The chimeric polypeptide of claim 16, wherein said second portion comprises a V3-loop selected from the group consisting of a HXB V3-loop, an 8x V3-loop, a BaL V3-loop, a YU-2 V3-loop, and an 89.6 V3-loop.

18. A composition comprising a CD4-independent HIV-1 Env comprising a gp120 polypeptide comprising a stably exposed chemokine receptor binding site wherein said HIV-1 is more sensitive to antibody neutralization than an otherwise identical HIV-1 which does not comprise a stably exposed chemokine receptor binding site.

19. A pharmaceutical composition comprising a CD4-independent HIV-1 Env protein, wherein said HIV-1 Env comprises at least one mutation causing the chemokine coreceptor binding site to be stably exposed.

20. The composition of claim 21, wherein said HIV-1 Env is HIV-1/IIIBx 8x.

21. A vaccine comprising an immunogenic dose of a CD4-independent HIV-1 Env.

22. The vaccine of claim 21, wherein said HIV-1 Env is selected from the group consisting of a HIV-1 Env polypeptide, a nucleic acid encoding HIV-1 Env, and a cell expressing HIV-1 Env.

23. A vector comprising the isolated nucleic acid of claim 1.

24. A vector comprising the isolated nucleic acid of claim 6.

25. A vector comprising the isolated nucleic acid of claim 7.

26. A cell comprising the isolated nucleic acid of claim 1.

27. A cell comprising the isolated nucleic acid of claim 6.

28. A cell comprising the isolated nucleic acid of claim 7.

29. A cell comprising the isolated polypeptide of claim 11.

30. A cell comprising the isolated polypeptide of claim 12.

31. A cell comprising the isolated polypeptide of claim 15.

32. A cell comprising the isolated polypeptide of claim 16.

33. A cell comprising the isolated polypeptide of claim 17.

34. A cell comprising the composition of claim 18.

35. A method of identifying an amino acid residue of an HIV-1 Env protein which is involved in CD4 independence, said method comprising obtaining a full-length env coding sequence from an Env clone which is CD4-independent and replacing at least a portion of the said env coding sequence with a coding sequence from an Env clone which is CD4-dependent to form a chimera, wherein when said chimera is CD4-dependent it is an

indication that said portion of said env coding sequence is involved in CD4-independence, thereby identifying an amino acid residue involved in CD4-independence.

36. A method of eliciting an immune response to a HIV-1 chemokine receptor binding site in a mammal, said method comprising administering an immunogenic dose of a CD4-independent HIV-1 Env protein to a mammal, wherein said protein comprises a stably exposed chemokine receptor binding site, thereby eliciting an immune response to a HIV-1 chemokine receptor binding site in said mammal.

37. A method of identifying a compound which affects exposure of an HIV-1 gp120 chemokine receptor binding site, said method comprising contacting a cell with said compound prior to or contemporaneous with contacting said cell with a labeled gp120 with or without pre-incubation of said gp120 with soluble CD4, measuring the amount of label bound to said cell, and comparing the amount of label bound to said cell contacted with said compound to the amount of label bound to an otherwise identical cell not contacted with said compound, wherein a higher or lower amount of label bound to said cell contacted with said compound compared with the amount of label bound to said otherwise identical cell not contacted with said compound, is an indication that said compound affects exposure of an HIV-1 gp120 chemokine receptor binding site.

38. A method of identifying a small-molecule which inhibits binding of an HIV-1 gp120, using its chemokine receptor binding site, to a chemokine receptor, said method comprising contacting a cell with said molecule prior to or contemporaneous with contacting said cell with labeled gp120 with or without pre-incubation of said gp120 with soluble CD4, measuring the amount of label bound to said cell, and comparing the amount of label bound to said cell contacted with said molecule with the amount of label bound to an otherwise identical cell not contacted with said molecule, wherein a lower amount of label bound to said cell contacted with said molecule compared with the amount of label bound to said otherwise identical cell not contacted with said molecule, is an indication that said molecule inhibits binding of an HIV-1 gp120 using its chemokine receptor binding site to a chemokine receptor.

39. A method of producing a CD4-independent chimeric HIV-1 Env clone comprising a variable chemokine receptor binding site, said method comprising replacing the hypervariable V3-loop of the CD4-independent Env clone with the V3 loop of another HIV-1, wherein said V3-loop of another HIV-1 comprises a different chemokine receptor binding site than that of said CD4-independent Env clone.

40. The method of claim 39, wherein said CD4-independent clone is selected from the group consisting of HIV-1/IIIBx, and HIV-1/IIIBx 8x.

41. The method of claim 40, wherein said V3-loop from another HIV-1 is selected from the group consisting of a V3-loop from HIV-1/BaL, a V3-loop from HIV-1/YU-2, a V3-loop from HIV-1/ADA, and a V3-loop from HIV-1/89.6.

42. A method of inhibiting HIV-1 gp120 binding, using its chemokine receptor binding site, to a chemokine receptor, said method comprising contacting said gp120 with a small-molecule identified using the method of claim 37, thereby inhibiting HIV-1 gp120 binding, using its chemokine receptor binding site, to a chemokine receptor.

43. A method of inhibiting HIV-1 infection of a cell, said method comprising contacting said cell with a small-molecule which inhibits binding of an HIV-1 gp120 using its chemokine receptor binding site to a chemokine receptor, wherein said small-molecule is identified using the method of claim 38, thereby inhibiting HIV-1 infection of a cell.

44. A composition comprising a CD4-independent HIV-1 Env and at least one compound used to treat HIV infection in a pharmaceutically suitable carrier.

45. The composition of claim 44, wherein said HIV-1 Env is selected from the group consisting of a HIV-1 Env polypeptide, a nucleic acid encoding HIV-1 Env, and a cell expressing HIV-1 env.

46. The composition of claim 44, wherein said compound used to treat HIV infection is selected from the group consisting of a protease inhibitor, a reverse transcriptase nucleoside analog inhibitor, a reverse transcriptase non-nucleoside analog inhibitor, an interferon, AZT, interleukin-2, and a cytokine.

47. A method of treating HIV-1 infection in a human, said method comprising administering an immunogenic dose of a CD4-independent HIV-1 Env to an HIV-1 infected human, thereby treating HIV-1 infection in said

human.

48. The method of claim 47, wherein said HIV-1 Env is selected from the group consisting of a HIV-1 Env polypeptide, a nucleic acid encoding HIV-1 Env, and a cell expressing HIV-1 env.

49. The method of claim 48, said method further comprising administering a compound used to treat HIV infection.

50. The method of claim 49, wherein said compound used to treat HIV infection is selected from the group consisting of a protease inhibitor, a reverse transcriptase nucleoside analog inhibitor, a reverse transcriptase non-nucleoside analog inhibitor, an interferon, AZT, interleukin-2, and a cytokine.

51. The method of claim 50, wherein said compound is administered to said human before, during or after administration of said CD4-independent HIV-1 Env.

L1 ANSWER 4 OF 12 USPATFULL on STN

2003:10696 Methods and compositions for modulating the interaction between the APJ receptor and the HIV virus.

Doms, Robert W., Berwyn, PA, UNITED STATES
Faulds, Daryl, Mill Valley, CA, UNITED STATES
Hesselgesser, Joseph E., San Francisco, CA, UNITED STATES
Horuk, Richard, Belmont, CA, UNITED STATES
Mitrovic, Branislava, Walnut Creek, CA, UNITED STATES
Zhou, Yiqing, El Sobrante, CA, UNITED STATES
Trustees of the University of Pennsylvania, Philadelphia, PA (U.S. corporation)
US 2003008391 A1 20030109
APPLICATION: US 2002-166372 A1 20020611 (10)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:

1. A recombinant eukaryotic cell transformed with a polynucleotide encoding an APJ polypeptide or a polynucleotide encoding a CD4 polypeptide, wherein the cell coexpresses APJ and CD4 polypeptides.

2. A recombinant eukaryotic cell transformed with a polynucleotide encoding an APJ polypeptide and a polynucleotide encoding a CD4 polypeptide, wherein the cell coexpresses APJ and CD4 polypeptides.

3. A recombinant eukaryotic cell according to claim 1, wherein the cell is stably transformed.

4. A recombinant eukaryotic cell according to claim 2, wherein the cell is stably transformed both polynucleotides.

5. The cell as in any of claims 1-4, wherein the cell is a human cell.

6. The cell as in any of claims 1-4, wherein the cell is a non-human cell.

7. An antibody which specifically binds to an extracellular domain of APJ, wherein the antibody inhibits HIV infection of a target cell that coexpresses APJ and CD4 polypeptides.

8. An antibody which specifically binds to an extracellular domain of APJ, wherein the antibody inhibits membrane fusion between a first cell coexpressing APJ and CD4 polypeptides and second cell expressing an HIV env protein.

9. An antibody according to claim 7 or 8, wherein the antibody is a monoclonal antibody.

10. An antibody according to claim 9, wherein the antibody recognizes an epitope comprising an amino acid sequence corresponding to a portion of the first extracellular domain of APJ.

11. An antibody according to claim 10, wherein the amino acid sequence corresponding to a portion of the first extracellular domain of APJ comprises the amino acid sequence Asn-Tyr-Tyr-Gly (SEQ ID NO: 3).

12. An antibody according to claim 9, wherein the antibody recognizes an epitope comprising an amino acid sequence corresponding to a portion of the second extracellular domain of APJ.

13. A substantially purified peptide fragment of APJ, wherein the peptide inhibits HIV infection of a target cell that coexpresses APJ and CD4 polypeptides.

14. A substantially purified peptide fragment of APJ, wherein the peptide inhibits cell fusion between a first cell coexpressing APJ and CD4 polypeptides and a second cell expressing an HIV env protein.

15. A substantially purified peptide fragment of APJ according to claim 13 or 14, wherein the peptide fragment comprises an amino acid sequence corresponding to a portion of the first extracellular domain of APJ.

16. A substantially purified peptide fragment of APJ according to claim 15, wherein the amino acid sequence corresponding to a portion of the first extracellular domain of APJ comprises the amino acid sequence Asn-Tyr-Tyr-Gly (SEQ ID NO: 3).

17. A substantially purified peptide fragment of APJ according to claim 13 or 14, wherein the peptide fragment comprises an amino acid sequence corresponding to a portion of the second extracellular domain of APJ.

18. A method for identifying a compound that modulates interaction between an HIV virus and an APJ receptor comprising incubating a first cell line which coexpresses CD4 and APJ polypeptides with a second cell line which expresses an env protein under conditions which promote cell fusion, in the presence and absence of a test compound, and determining whether the presence of the test compound inhibits cell fusion between the first cell line and the second cell line.

19. A method according to claim 18, wherein cell fusion is determined by detection of a reporter molecule.

20. A method according to claim 19, wherein the reporter molecule is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme.

21. A method according to claim 19 wherein the reporter molecule is B-galactosidase or luciferase.

22. A method for identifying a compound that modulates interaction between an HIV virus and an APJ receptor comprising incubating a cell line which expresses CD4 and APJ polypeptides with a test virus carrying an env protein, in the presence and absence of a test compound, and determining whether the presence of the test compound inhibits infection of the cell line by the test virus.

23. A method according to claim 22, wherein infection is determined by detection of a reporter molecule.

24. A method according to claim 23, wherein the reporter molecule is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme.

25. A method according to claim 23, wherein the reporter molecule is B-galactosidase or luciferase.

26. A method of inhibiting HIV infection of a target cell expressing an APJ and CD4 polypeptides comprising contacting the target cell with an effective amount of a APJ binding or blocking agent.

27. The method of claim 26, wherein the agent is an anti-APJ antibody or epitope binding fragment thereof.

28. The method of claim 27, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

29. The method of claim 26, wherein said contacting is accomplished by in vivo administration to a subject.

30. The method of claim 26, wherein the agent is a peptide fragment of APJ.

31. A method of treating a subject having an HIV-related disorder associated with expression of APJ comprising administering an agent that suppresses APJ to the subject.

32. The method of claim 31, wherein the agent is an anti-APJ antibody.

33. The method of claim 31, wherein the agent is an antisense polynucleotide that hybridizes to an APJ polynucleotide.

34. The method of claim 31, wherein the agent is introduced into a cell using a carrier.

35. The method of claim 34, wherein the carrier is a vector.

36. A method of treating a subject having or at risk of having an HIV infection or related disorder, comprising administering a therapeutically effective amount of an anti-APJ antibody or a peptide fragment to the subject.

37. A method according to claim 36, wherein the subject is a fetus.

38. A transgenic non-human animal having a phenotype characterized by expression of APJ polypeptide and CD4 polypeptide otherwise not naturally occurring in the animal, wherein the phenotype is conferred by a transgene contained in the somatic cells and germ cells of the animal, and wherein the transgene comprises a polynucleotide encoding an APJ polypeptide and a polynucleotide encoding a CD4 polypeptide.

L1 ANSWER 5 OF 12 USPATFULL on STN
2003:10681 Methods and compositions for modulating the interaction between the APJ receptor and the HIV virus.
Doms, Robert W., Berwyn, PA, UNITED STATES
Faulds, Daryl, Mill Valley, CA, UNITED STATES
Hesselgesser, Joseph E., San Francisco, CA, UNITED STATES
Horuk, Richard, Belmont, CA, UNITED STATES
Mitrovic, Branislava, Walnut Creek, CA, UNITED STATES
Zhou, Yiqing, El Sobrante, CA, UNITED STATES
Trustees of the University of Pennsylvania, Philadelphia, PA (U.S. corporation)
US 2003008376 A1 20030109
APPLICATION: US 2002-166357 A1 20020611 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A recombinant eukaryotic cell transformed with a polynucleotide encoding an APJ polypeptide or a polynucleotide encoding a CD4 polypeptide, wherein the cell coexpresses APJ and CD4 polypeptides.

2. A recombinant eukaryotic cell transformed with a polynucleotide encoding an APJ polypeptide and a polynucleotide encoding a CD4 polypeptide, wherein the cell coexpresses APJ and CD4 polypeptides.

3. A recombinant eukaryotic cell according to claim 1, wherein the cell is stably transformed.

4. A recombinant eukaryotic cell according to claim 2, wherein the cell is stably transformed both polynucleotides.

5. The cell as in any of claims 1-4, wherein the cell is a human cell.

6. The cell as in any of claims 1-4, wherein the cell is a non-human cell.

7. An antibody which specifically binds to an extracellular domain of APJ, wherein the antibody inhibits HIV infection of a target cell that coexpresses APJ and CD4 polypeptides.

8. An antibody which specifically binds to an extracellular domain of APJ, wherein the antibody inhibits membrane fusion between a first cell coexpressing APJ and CD4 polypeptides and second cell expressing an HIV env protein.

9. An antibody according to claim 7 or 8, wherein the antibody is a monoclonal antibody.

10. An antibody according to claim 9, wherein the antibody recognizes an epitope comprising an amino acid sequence corresponding to a portion of the first extracellular domain of APJ.

11. An antibody according to claim 10, wherein the amino acid sequence corresponding to a portion of the first extracellular domain of APJ comprises the amino acid sequence Asn-Tyr-Tyr-Gly (SEQ ID NO: 3).

12. An antibody according to claim 9, wherein the antibody recognizes an epitope comprising an amino acid sequence corresponding to a portion of the second extracellular domain of APJ.

13. A substantially purified peptide fragment of APJ, wherein the peptide inhibits HIV infection of a target cell that coexpresses APJ and CD4 polypeptides.

14. A substantially purified peptide fragment of APJ, wherein the peptide inhibits cell fusion between a first cell coexpressing APJ and CD4 polypeptides and a second cell expressing an HIV env protein.

15. A substantially purified peptide fragment of APJ according to claim 13 or 14, wherein the peptide fragment comprises an amino acid sequence corresponding to a portion of the first extracellular domain of APJ.

16. A substantially purified peptide fragment of APJ according to claim 15, wherein the amino acid sequence corresponding to a portion of the first extracellular domain of APJ comprises the amino acid sequence Asn-Tyr-Tyr-Gly (SEQ ID NO:3).

17. A substantially purified peptide fragment of APJ according to claim 13 or 14, wherein the peptide fragment comprises an amino acid, sequence corresponding to a portion of the second extracellular domain of APJ.

18. A method for identifying a compound that modulates interaction between an HIV virus and an APJ receptor comprising incubating a first cell line which coexpresses CD4 and APJ polypeptides with a second cell line which expresses an env protein under conditions which promote cell fusion, in the presence and absence of a test compound, and determining whether the presence of the test compound inhibits cell fusion between the first cell line and the second cell line.

19. A method according to claim 18, wherein cell fusion is determined by detection of a reporter molecule.

20. A method according to claim 19, wherein the reporter molecule is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme.

21. A method according to claim 19 wherein the reporter molecule is B-galactosidase or luciferase.

22. A method for identifying a compound that modulates interaction between an HIV virus and an APJ receptor comprising incubating a cell line which expresses CD4 and APJ polypeptides with a test virus carrying an env protein, in the presence and absence of a test compound, and determining whether the presence of the test compound inhibits infection of the cell line by the test virus.

23. A method according to claim 22, wherein infection is determined by detection of a reporter molecule.

24. A method according to claim 23, wherein the reporter molecule is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme.

25. A method according to claim 23, wherein the reporter molecule is B-galactosidase or luciferase.

26. A method of inhibiting HIV infection of a target cell expressing an APJ and CD4 polypeptides comprising contacting the target cell with an effective amount of a APJ binding or blocking agent.

27. The method of claim 26, wherein the agent is an anti-APJ antibody or epitope binding fragment thereof.

28. The method of claim 27, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

29. The method of claim 26, wherein said contacting is accomplished by in vivo administration to a subject.

30. The method of claim 26, wherein the agent is a peptide fragment of APJ.

31. A method of treating a subject having an HIV-related disorder associated with expression of APJ comprising administering an agent that suppresses APJ to the subject.

32. The method of claim 31, wherein the agent is an anti-APJ antibody.

33. The method of claim 31, wherein the agent is an antisense polynucleotide that hybridizes to an APJ polynucleotide.

34. The method of claim 31, wherein the agent is introduced into a cell using a carrier.

35. The method of claim 34, wherein the carrier is a vector.

36. A method of treating a subject having or at risk of having an HIV infection or related disorder, comprising administering a therapeutically effective amount of an anti-APJ antibody or a peptide

fragment to the subject.

37. A method according to claim 36, wherein the subject is a fetus.

38. A transgenic non-human animal having a phenotype characterized by expression of APJ polypeptide and CD4 polypeptide otherwise not naturally occurring in the animal, wherein the phenotype is conferred by a transgene contained in the somatic cells and germ cells of the animal, and wherein the transgene comprises a polynucleotide encoding an APJ polypeptide and a polynucleotide encoding a CD4 polypeptide.

L1 ANSWER 6 OF 12 USPATFULL on STN

2003:10585 Methods and compositions for modulating the interaction between the APJ receptor and the HIV virus.

Doms, Robert W., Berwyn, PA, UNITED STATES
Faulds, Daryl, Mill Valley, CA, UNITED STATES
Hesselgesser, Joseph E., San Francisco, CA, UNITED STATES
Horuk, Richard, Belmont, CA, UNITED STATES
Mitrovic, Branislava, Walnut Creek, CA, UNITED STATES
Zhou, Yiqing, El Sobrante, CA, UNITED STATES
Trustees of the University of Pennsylvania, Philadelphia, PA (U.S. corporation)

US 2003008279 A1 20030109
APPLICATION: US 2002-166113 A1 20020611 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:

1. A recombinant eukaryotic cell transformed with a polynucleotide encoding an APJ polypeptide or a polynucleotide encoding a CD4 polypeptide, wherein the cell coexpresses APJ and CD4 polypeptides.

2. A recombinant eukaryotic cell transformed with a polynucleotide encoding an APJ polypeptide and a polynucleotide encoding a CD4 polypeptide, wherein the cell coexpresses APJ and CD4 polypeptides.

3. A recombinant eukaryotic cell according to claim 1, wherein the cell is stably transformed.

4. A recombinant eukaryotic cell according to claim 2, wherein the cell is stably transformed both polynucleotides.

5. The cell as in any of claims 1-4, wherein the cell is a human cell.

6. The cell as in any of claims 1-4, wherein the cell is a non-human cell.

7. An antibody which specifically binds to an extracellular domain of APJ, wherein the antibody inhibits HIV infection of a target cell that coexpresses APJ and CD4 polypeptides.

8. An antibody which specifically binds to an extracellular domain of APJ, wherein the antibody inhibits membrane fusion between a first cell coexpressing APJ and CD4 polypeptides and second cell expressing an HIV env protein.

9. An antibody according to claim 7 or 8, wherein the antibody is a monoclonal antibody.

10. An antibody according to claim 9, wherein the antibody recognizes an epitope comprising an amino acid sequence corresponding to a portion of the first extracellular domain of APJ.

11. An antibody according to claim 10, wherein the amino acid sequence corresponding to a portion of the first extracellular domain of APJ comprises the amino acid sequence Asn-Tyr-Tyr-Gly (SEQ ID NO: 3).

12. An antibody according to claim 9, wherein the antibody recognizes an epitope comprising an amino acid sequence corresponding to a portion of the second extracellular domain of APJ.

13. A substantially purified peptide fragment of APJ, wherein the peptide inhibits HIV infection of a target cell that coexpresses APJ and CD4 polypeptides.

14. A substantially purified peptide fragment of APJ, wherein the peptide inhibits cell fusion between a first cell coexpressing APJ and CD4 polypeptides and a second cell expressing an HIV env protein.

15. A substantially purified peptide fragment of APJ according to claim 13 or 14, wherein the peptide fragment comprises an amino acid sequence corresponding to a portion of the first extracellular domain of APJ.

16. A substantially purified peptide fragment of APJ according to claim 15, wherein the amino acid sequence corresponding to a portion of the first extracellular domain of APJ comprises the amino acid sequence Asn-Tyr-Tyr-Gly (SEQ ID NO:3).

17. A substantially purified peptide fragment of APJ according to claim 13 or 14, wherein the peptide fragment comprises an amino acid sequence corresponding to a portion of the second extracellular domain of APJ.

18. A method for identifying a compound that modulates interaction between an HIV virus and an APJ receptor comprising incubating a first cell line which coexpresses CD4 and APJ polypeptides with a second cell line which expresses an env protein under conditions which promote cell fusion, in the presence and absence of a test compound, and determining whether the presence of the test compound inhibits cell fusion between the first cell line and the second cell line.

19. A method according to claim 18, wherein cell fusion is determined by detection of a reporter molecule.

20. A method according to claim 19, wherein the reporter molecule is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme.

21. A method according to claim 19 wherein the reporter molecule is B-galactosidase or luciferase.

22. A method for identifying a compound that modulates interaction between an HIV virus and an APJ receptor comprising incubating a cell line which expresses CD4 and APJ polypeptides with a test virus carrying an env protein, in the presence and absence of a test compound, and determining whether the presence of the test compound inhibits infection of the cell line by the test virus.

23. A method according to claim 22, wherein infection is determined by detection of a reporter molecule.

24. A method according to claim 23, wherein the reporter molecule is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme.

25. A method according to claim 23, wherein the reporter molecule is B-galactosidase or luciferase.

26. A method of inhibiting HIV infection of a target cell expressing an APJ and CD4 polypeptides comprising contacting the target cell with an effective amount of a APJ binding or blocking agent.

27. The method of claim 26, wherein the agent is an anti-APJ antibody or epitope binding fragment thereof.

28. The method of claim 27, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

29. The method of claim 26, wherein said contacting is accomplished by in vivo administration to a subject.

30. The method of claim 26, wherein the agent is a peptide fragment of APJ.

31. A method of treating a subject having an HIV-related disorder associated with expression of APJ comprising administering an agent that suppresses APJ to the subject.

32. The method of claim 31, wherein the agent is an anti-APJ antibody.

33. The method of claim 31, wherein the agent is an antisense polynucleotide that hybridizes to an APJ polynucleotide.

34. The method of claim 31, wherein the agent is introduced into a cell using a carrier.

35. The method of claim 34, wherein the carrier is a vector.

36. A method of treating a subject having or at risk of having an HIV infection or related disorder, comprising administering a therapeutically effective amount of an anti-APJ antibody or a peptide fragment to the subject.

37. A method according to claim 36, wherein the subject is a fetus.

expression of APJ polypeptide and CD4 polypeptide otherwise not naturally occurring in the animal, wherein the phenotype is conferred by a transgene contained in the somatic cells and germ cells of the animal, and wherein the transgene comprises a polynucleotide encoding an APJ polypeptide and a polynucleotide encoding a CD4 polypeptide.

L1 ANSWER 7 OF 12 USPATFULL on STN

2003:3412 Methods and compositions for modulating the interaction between the APJ receptor and the HIV virus.

Doms, Robert W., Berwyn, PA, UNITED STATES
Faulds, Daryl, Mill Valley, CA, UNITED STATES
Hesselgesser, Joseph E., San Francisco, CA, UNITED STATES
Horuk, Richard, Belmont, CA, UNITED STATES
Mitrovic, Branislava, Walnut Creek, CA, UNITED STATES
Zhou, Yiqing, El Sobrante, CA, UNITED STATES
Trustees of the University of Pennsylvania, Philadelphia, PA (U.S. corporation)
US 2003003447 A1 20030102
APPLICATION: US 2002-166359 A1 20020611 (10)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:

1. A recombinant eukaryotic cell transformed with a polynucleotide encoding an APJ polypeptide or a polynucleotide encoding a CD4 polypeptide, wherein the cell coexpresses APJ and CD4 polypeptides.

2. A recombinant eukaryotic cell transformed with a polynucleotide encoding an APJ polypeptide and a polynucleotide encoding a CD4 polypeptide, wherein the cell coexpresses APJ and CD4 polypeptides.

3. A recombinant eukaryotic cell according to claim 1, wherein the cell is stably transformed.

4. A recombinant eukaryotic cell according to claim 2, wherein the cell is stably transformed both polynucleotides.

5. The cell as in any of claims 1-4, wherein the cell is a human cell.

6. The cell as in any of claims 1-4, wherein the cell is a non-human cell.

7. An antibody which specifically binds to an extracellular domain of APJ, wherein the antibody inhibits HIV infection of a target cell that coexpresses APJ and CD4 polypeptides.

8. An antibody which specifically binds to an extracellular domain of APJ, wherein the antibody inhibits membrane fusion between a first cell coexpressing APJ and CD4 polypeptides and second cell expressing an HIV env protein.

9. An antibody according to claim 7 or 8, wherein the antibody is a monoclonal antibody.

10. An antibody according to claim 9, wherein the antibody recognizes an epitope comprising an amino acid sequence corresponding to a portion of the first extracellular domain of APJ.

11. An antibody according to claim 10, wherein the amino acid sequence corresponding to a portion of the first extracellular domain of APJ comprises the amino acid sequence Asn-Tyr-Tyr-Gly (SEQ ID NO: 3).

12. An antibody according to claim 9, wherein the antibody recognizes an epitope comprising an amino acid sequence corresponding to a portion of the second extracellular domain of APJ.

13. A substantially purified peptide fragment of APJ, wherein the peptide inhibits HIV infection of a target cell that coexpresses APJ and CD4 polypeptides.

14. A substantially purified peptide fragment of APJ, wherein the peptide inhibits cell fusion between a first cell coexpressing APJ and CD4 polypeptides and a second cell expressing an HIV env protein.

15. A substantially purified peptide fragment of APJ according to claim 13 or 14, wherein the peptide fragment comprises an amino acid sequence corresponding to a portion of the first extracellular domain of APJ.

16. A substantially purified peptide fragment of APJ according to claim 15, wherein the amino acid sequence corresponding to a portion of the first extracellular domain of APJ comprises the amino acid sequence Asn-Tyr-Tyr-Gly (SEQ ID NO:3).

17. A substantially purified peptide fragment of APJ according to claim 13 or 14, wherein the peptide fragment comprises an amino acid sequence corresponding to a portion of the second extracellular domain of APJ.

18. A method for identifying a compound that modulates interaction between an HIV virus and an APJ receptor comprising incubating a first cell line which coexpresses CD4 and APJ polypeptides with a second cell line which expresses an env protein under conditions which promote cell fusion, in the presence and absence of a test compound, and determining whether the presence of the test compound inhibits cell fusion between the first cell line and the second cell line.

19. A method according to claim 18, wherein cell fusion is determined by detection of a reporter molecule.

20. A method according to claim 19, wherein the reporter molecule is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme.

21. A method according to claim 19 wherein the reporter molecule is B-galactosidase or luciferase.

22. A method for identifying a compound that modulates interaction between an HIV virus and an APJ receptor comprising incubating a cell line which expresses CD4 and APJ polypeptides with a test virus carrying an env protein, in the presence and absence of a test compound, and determining whether the presence of the test compound inhibits infection of the cell line by the test virus.

23. A method according to claim 22, wherein infection is determined by detection of a reporter molecule.

24. A method according to claim 23, wherein the reporter molecule is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme.

25. A method according to claim 23, wherein the reporter molecule is B-galactosidase or luciferase.

26. A method of inhibiting HIV infection of a target cell expressing an APJ and CD4 polypeptides comprising contacting the target cell with an effective amount of a APJ binding or blocking agent.

27. The method of claim 26, wherein the agent is an anti-APJ antibody or epitope binding fragment thereof.

28. The method of claim 27, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

29. The method of claim 26, wherein said contacting is accomplished by in vivo administration to a subject.

30. The method of claim 26, wherein the agent is a peptide fragment of APJ.

31. A method of treating a subject having an HIV-related disorder associated with expression of APJ comprising administering an agent that suppresses APJ to the subject.

32. The method of claim 31, wherein the agent is an anti-APJ antibody.

33. The method of claim 31, wherein the agent is an antisense polynucleotide that hybridizes to an APJ polynucleotide.

34. The method of claim 31, wherein the agent is introduced into a cell using a carrier.

35. The method of claim 34, wherein the carrier is a vector.

36. A method of treating a subject having or at risk of having an HIV infection or related disorder, comprising administering a therapeutically effective amount of an anti-APJ antibody or a peptide fragment to the subject.

37. A method according to claim 36, wherein the subject is a fetus.

38. A transgenic non-human animal having a phenotype characterized by expression of APJ polypeptide and CD4 polypeptide otherwise not naturally occurring in the animal, wherein the phenotype is conferred by a transgene contained in the somatic cells and germ cells of the animal,

and wherein the transgene comprises a polynucleotide encoding an ... polypeptide and a polynucleotide encoding a CD4 polypeptide.

L1 ANSWER 8 OF 12 USPATFULL on STN
2002:323081 Lipoparticle comprising a protein and methods of making and using the same.
Doms, Robert W., Berwyn, PA, UNITED STATES
Rucker, Joseph, Philadelphia, PA, UNITED STATES
Hoffman, Trevor L., Lansdowne, PA, UNITED STATES
Bates, Paul, Swarthmore, PA, UNITED STATES
Hoxie, James A., Berwyn, PA, UNITED STATES
The Trustees of the University of Pennsylvania (U.S. corporation)
US 2002183247 A1 20021205
APPLICATION: US 2001-32311 A1 20011221 (10)
PRIORITY: US 1997-47226P 19970520 (60)
US 2000-257988P 20001222 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An isolated lipoparticle comprising a multiple membrane spanning protein wherein said protein is not CD63.

2. The lipoparticle of claim 1, wherein said protein is capable of binding with a ligand under conditions wherein said ligand would bind with an otherwise identical protein present on a cell membrane.

3. The isolated lipoparticle of claim 1, wherein said lipoparticle is a virus.

4. The isolated lipoparticle of claim 3, wherein said virus is a membrane-enveloped virus.

5. The isolated lipoparticle of claim 4, wherein said membrane-enveloped virus is a retrovirus.

6. The isolated lipoparticle of claim 4, wherein said virus is selected from the group consisting of a murine leukemia virus, a human immunodeficiency virus, a rabies virus, a Rous sarcoma virus, and a vesicular stomatitis virus.

7. The isolated lipoparticle of claim 1, wherein said protein is selected from the group consisting of a G-protein coupled receptor, a transporter protein, and an ion channel protein.

8. The isolated lipoparticle of claim 1, wherein said protein is selected from the group consisting of CCR5, CXCR4,, MCAT-1, CXCR2, CXCR3, mu-opioid receptor, and KCNH2 potassium channel protein.

9. A composition comprising an isolated lipoparticle attached to a sensor surface, said lipoparticle further comprising a membrane spanning protein.

10. The composition of claim 9, wherein said protein is selected from the group consisting of a transport protein, a G-protein coupled receptor, an ion channel protein, a type I membrane protein, and a type II membrane protein.

11. The composition of claim 10, wherein said G-protein coupled receptor is selected from the group consisting of a mu-opioid receptor, a CXCR2, CXCR3, CXCR4, a CCR5, a CCR8, a XCR1, and a CX3CR1.

12. The composition of claim 10, wherein said ion channel protein is selected from the group consisting of KCNH2 potassium channel protein, Kv1.3 potassium channel protein, and CFTR protein.

13. The composition of claim 10, wherein said transporter protein is selected from a group consisting of a glucose transporter protein and an amino acid transporter protein.

14. The composition of claim 10, wherein said type I membrane protein is selected from the group consisting of CD4, Tva, and neuropilin-2.

15. The composition of claim 10, wherein said type II membrane protein comprises DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN).

16. The composition of claim 9, wherein said lipoparticle is a virus.

17. The composition of claim 16, wherein said virus is a membrane-enveloped virus.

18. The composition of claim 17, wherein said membrane-enveloped virus is a retrovirus.

19. The composition of claim 17, wherein said virus is selected from the group consisting of a murine leukemia virus, a human immunodeficiency virus, a rabies virus, a Rous sarcoma virus, and a vesicular stomatitis virus.

20. The composition of claim 9, wherein said lipoparticle further comprises a plastic bead core to form a proteoliposome.

21. The composition of claim 9, wherein said sensor comprises a microfluidic device.

22. The composition of claim 21, wherein said microfluidic device is a biosensor.

23. The composition of claim 22, wherein said biosensor is an optical biosensor.

24. The composition of claim 23, wherein said optical biosensor measures surface plasmon resonance (SPR).

25. The composition of claim 23, wherein said surface is located on a biosensor chip.

26. The composition of claim 25, wherein said biosensor chip is selected from the group consisting of a gold coated biosensor chip, a gold and dextran coated biosensor chip, and a derivatized gold biosensor chip.

27. A method of assessing the binding interaction of a membrane spanning protein with a ligand, said method comprising (a) producing a lipoparticle comprising a membrane spanning protein; (b) attaching said lipoparticle to a substrate; (c) contacting said protein present on said lipoparticle with a ligand of said protein; and (d) detecting any change in said substrate compared with any change in an otherwise identical substrate wherein said protein present on said lipoparticle is not contacted with said ligand, wherein detecting a change in said substrate wherein said protein present on said lipoparticle is contacted with said ligand compared with said otherwise identical substrate wherein said protein present on said lipoparticle is not contacted with said ligand assesses said binding interaction of said protein with said ligand.

28. The method of claim 27, wherein said detecting in (d) is performed using a microfluidic device and said substrate is a sensor surface.

29. The method of claim 28, wherein said microfluidic device is a biosensor device.

30. The method of claim 27, wherein said biosensor device comprises a microchannel or a microwell.

31. The method of claim 29, wherein said biosensor is an optical biosensor.

32. The method of claim 31, wherein said optical biosensor is a surface plasmon resonance biosensor device.

33. A method of identifying a potential ligand of a membrane protein, said method comprising (a) attaching a lipoparticle comprising a membrane protein to a surface; (b) contacting said protein with a test ligand; and (c) comparing said surface comprising said lipoparticle comprising said protein contacted with said test ligand with an otherwise identical surface comprising an otherwise identical lipoparticle comprising a protein not contacted with said test ligand, wherein a difference between said surface comprising said lipoparticle comprising a protein contacted with said test ligand compared with said otherwise identical surface comprising said otherwise identical lipoparticle comprising said protein not contacted with said test ligand is an indication that said ligand is a potential ligand of said protein.

34. The method of claim 33, wherein said comparing in (c) is performed using a microfluidic device.

35. The method of claim 34, wherein said microfluidic device is a biosensor device.

36. The method of claim 33, wherein said protein is selected from a multiple membrane spanning protein and a single membrane spanning protein.

37. The method of claim 33, wherein said multiple membrane spanning protein is selected from the group consisting of a G-coupled protein receptor (GCPR), a transporter, and an ion channel.

38. The method of claim 36, wherein said single membrane spanning protein is selected from the group consisting of a type I membrane protein and a type II membrane protein.

39. The method of claim 33, wherein said test ligand is selected from the group consisting of a protein and a chemical compound.

40. The method of claim 39, wherein said protein is an antibody.

41. A ligand identified by the method of claim 33.

42. A method of identifying a compound that affects binding between a ligand and a membrane protein receptor, said method comprising (a) attaching a lipoparticle comprising a membrane protein to a surface; (b) contacting said protein with a known ligand under conditions wherein said protein specifically binds with said ligand; (c) contacting said lipoparticle of (b) with a test compound; and (d) comparing said surface comprising said lipoparticle contacted with said test compound with an otherwise identical surface comprising an otherwise identical lipoparticle not contacted with said test compound, wherein a difference between said surface comprising said lipoparticle contacted with said test compound compared with said otherwise identical surface comprising said otherwise identical lipoparticle not contacted with said test compound is an indication that said test compound affects between said ligand and said membrane protein receptor.

43. A kit for assessing the binding interaction of a membrane spanning protein with a ligand, said kit comprising a lipoparticle comprising a membrane spanning protein and a substrate, said kit further comprising an applicator, and an instructional material for the use thereof.

44. The kit of claim 43, said kit further comprising a ligand of said protein.

45. A kit for identifying a potential ligand of a membrane protein, said kit comprising a lipoparticle comprising a membrane protein and a surface, said kit further comprising an applicator, and an instructional material for the use thereof.

46. The kit of claim 45, said kit further comprising a test ligand.

47. A kit for identifying a compound that affects binding between a ligand and a membrane protein receptor, said kit comprising a lipoparticle comprising a membrane protein and a surface, said kit further comprising an applicator, and an instructional material for the use thereof.

48. The kit of claim 47, said kit further comprising a test compound.

49. The kit of claim 47, said kit further comprising a known ligand of said membrane protein.

L1 ANSWER 9 OF 12 USPATFULL on STN
2002:175297 CD4-independent HIV envelope proteins as vaccines and therapeutics.
Hoxie, James A., Berwyn, PA, United States
LaBranche, Celia C., Chapel Hill, NC, United States
Doms, Robert W., Berwyn, PA, United States
Hoffman, Trevor L., Lansdowne, PA, United States
The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)Duke University, Durham, NC, United States (U.S. corporation)
US 6420545 B1 20020716
APPLICATION: US 1999-337387 19990622 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An isolated nucleic acid encoding a CD4-independent HIV env having the nucleotide sequence of SEQ ID NO:4.

2. A vector comprising the isolated nucleic acid of claim 1.

3. A cell comprising the isolated nucleic acid of claim 1.

L1 ANSWER 10 OF 12 USPATFULL on STN
2002:120498 METHODS AND COMPOSITIONS FOR MODULATING THE INTERACTION BETWEEN THE APJ RECEPTOR AND THE HIV VIRUS.
DOMS, ROBERT W., BERWYN, PA, UNITED STATES
FAULDS, DARYL, MILL VALLEY, CA, UNITED STATES
HESSELGESSER, JOSEPH E., SAN FRANCISCO, CA, UNITED STATES
HORUK, RICHARD, BELMONT, CA, UNITED STATES

ZHOU, YIQING, EL SOBRANTE, CA, UNITED STATES
US 2002062488 A1 20020523
APPLICATION: US 1998-149045 A1 19980908 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A recombinant eukaryotic cell transformed with a polynucleotide encoding an APJ polypeptide or a polynucleotide encoding a CD4 polypeptide, wherein the cell coexpresses APJ and CD4 polypeptides.

2. A recombinant eukaryotic cell transformed with a polynucleotide encoding an APJ polypeptide and a polynucleotide encoding a CD4 polypeptide, wherein the cell coexpresses APJ and CD4 polypeptides.

3. A recombinant eukaryotic cell according to claim 1, wherein the cell is stably transformed.

4. A recombinant eukaryotic cell according to claim 2, wherein the cell is stably transformed both polynucleotides.

5. The cell as in any of claims 1-4, wherein the cell is a human cell.

6. The cell as in any of claims 1-4, wherein the cell is a non-human cell.

7. An antibody which specifically binds to an extracellular domain of APJ, wherein the antibody inhibits HIV infection of a target cell that coexpresses APJ and CD4 polypeptides.

8. An antibody which specifically binds to an extracellular domain of APJ, wherein the antibody inhibits membrane fusion between a first cell coexpressing APJ and CD4 polypeptides and second cell expressing an HIV env protein.

9. An antibody according to claim 7 or 8, wherein the antibody is a monoclonal antibody.

10. An antibody according to claim 9, wherein the antibody recognizes an epitope comprising an amino acid sequence corresponding to a portion of the first extracellular domain of APJ.

11. An antibody according to claim 10, wherein the amino acid sequence corresponding to a portion of the first extracellular domain of APJ comprises the amino acid sequence Asn-Tyr-Tyr-Gly (SEQ ID NO: 3).

12. An antibody according to claim 9, wherein the antibody recognizes an epitope comprising an amino acid sequence corresponding to a portion of the second extracellular domain of APJ.

13. A substantially purified peptide fragment of APJ, wherein the peptide inhibits HIV infection of a target cell that coexpresses APJ and CD4 polypeptides.

14. A substantially purified peptide fragment of APJ, wherein the peptide inhibits cell fusion between a first cell coexpressing APJ and CD4 polypeptides and a second cell expressing an HIV env protein.

15. A substantially purified peptide fragment of APJ according to claim 13 or 14, wherein the peptide fragment comprises an amino acid sequence corresponding to a portion of the first extracellular domain of APJ.

16. A substantially purified peptide fragment of APJ according to claim 15, wherein the amino acid sequence corresponding to a portion of the first extracellular domain of APJ comprises the amino acid sequence Asn-Tyr-Tyr-Gly (SEQ ID NO:3).

17. A substantially purified peptide fragment of APJ according to claim 13 or 14, wherein the peptide fragment comprises an amino acid sequence corresponding to a portion of the second extracellular domain of APJ.

18. A method for identifying a compound that modulates interaction between an HIV virus and an APJ receptor comprising incubating a first cell line which coexpresses CD4 and APJ polypeptides with a second cell line which expresses an env protein under conditions which promote cell fusion, in the presence and absence of a test compound, and determining whether the presence of the test compound inhibits cell fusion between the first cell line and the second cell line.

19. A method according to claim 18, wherein cell fusion is determined by detection of a reporter molecule.

20. A method according to claim 19, wherein the reporter molecule is

selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme.

21. A method according to claim 19 wherein the reporter molecule is B-galactosidase or luciferase.

22. A method for identifying a compound that modulates interaction between an HIV virus and an APJ receptor comprising incubating a cell line which expresses CD4 and APJ polypeptides with a test virus carrying an env protein, in the presence and absence of a test compound, and determining whether the presence of the test compound inhibits infection of the cell line by the test virus.

23. A method according to claim 22, wherein infection is determined by detection of a reporter molecule.

24. A method according to claim 23, wherein the reporter molecule is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme.

25. A method according to claim 23, wherein the reporter molecule is B-galactosidase or luciferase.

26. A method of inhibiting HIV infection of a target cell expressing an APJ and CD4 polypeptides comprising contacting the target cell with an effective amount of a APJ binding or blocking agent.

27. The method of claim 26, wherein the agent is an anti-APJ antibody or epitope binding fragment thereof.

28. The method of claim 27, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

29. The method of claim 26, wherein said contacting is accomplished by in vivo administration to a subject.

30. The method of claim 26, wherein the agent is a peptide fragment of APJ.

31. A method of treating a subject having an HIV-related disorder associated with expression of APJ comprising administering an agent that suppresses APJ to the subject.

32. The method of claim 31, wherein the agent is an anti-APJ antibody.

33. The method of claim 31, wherein the agent is an antisense polynucleotide that hybridizes to an APJ polynucleotide.

34. The method of claim 31, wherein the agent is introduced into a cell using a carrier.

35. The method of claim 34, wherein the carrier is a vector.

36. A method of treating a subject having or at risk of having an HIV infection or related disorder, comprising administering a therapeutically effective amount of an anti-APJ antibody or a peptide fragment to the subject.

37. A method according to claim 36, wherein the subject is a fetus.

38. A transgenic non-human animal having a phenotype characterized by expression of APJ polypeptide and CD4 polypeptide otherwise not naturally occurring in the animal, wherein the phenotype is conferred by a transgene contained in the somatic cells and germ cells of the animal, and wherein the transgene comprises a polynucleotide encoding an APJ polypeptide and a polynucleotide encoding a CD4 polypeptide.

L1 ANSWER 11 OF 12 USPATFULL on STN
2001:4271 Oligomeric HIV-1 envelope glycoproteins.
Earl, Patricia L., ChevyChase, MD, United States
Broder, Christopher C., Rockville, MD, United States
Doms, Robert W., Berwyn, PA, United States
Moss, Bernard, Bethesda, MD, United States
The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 6171596 B1 20010109
APPLICATION: US 1998-70291 19980430 (9)
DOCUMENT TYPE: Patent; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An immunogenic composition comprising a pharmaceutically acceptable carrier and a recombinant uncleaved gp140 protein retaining its oligomeric structure so as that neutralizing antibodies against conformational epitopes of HIV-1 envelope proteins found on the oligomeric structure of said gp140 are produced in an immunized human, said gp140 protein being defined as a C-terminally truncated form of HIV-1 gp160 protein that is missing the gp41 transmembrane domain.

2. The immunogenic composition of claim 1, wherein said gp140 protein is obtained by running said gp14O through lectin chromatography followed by a sizings separation.

3. The immunogenic composition of claim 1, wherein said gp140 protein is obtained by running said gp140 through affinity chromatography with elution at pH8 followed by a sizing separation.

4. The immunogenic composition of claim 1, 2, or 3, wherein said gp140 protein is further defined as missing the gp120/gp41 cleaving site.

5. The immunogenic composition of claim 1, 2, or 3 wherein said gp140 protein is further defined as retaining the gp120/pg41 cleavage site.

L1 ANSWER 12 OF 12 USPATFULL on STN
2000:34204 Oligomeric HIV-1 envelope glycoproteins.
Earl, Patricia L., ChevyChase, MD, United States
Broder, Christopher C., Rockville, MD, United States
Doms, Robert W., Berwyn, PA, United States
Moss, Bernard, Bethesda, MD, United States
United States of America, as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 6039957 20000321
APPLICATION: US 1997-805889 19970303 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method of producing neutralizing antibodies against conformational epitopes of HIV-1 envelope proteins in a human, comprising: administering to a human a recombinant uncleaved gp140 protein retaining its oligomeric structure so that said human produces neutralizing antibodes against conformational epitopes of HIV-1 envelope proteins found on the oligomeric structure of said gp 140, said gp140 protein being defined as a C-terminally truncated form of HIV-1 gp160 protein that is missing the gp41 transmembrane domain.

2. The method of claim 1 wherein said administering step is selected from the group consisting of intradermal, intramuscular, intraperitoneal and intravenous administration.

3. The method of claim 1 further comprising the step of obtaining said gp140 protein by running said gp140 through lectin chromatography followed by a sizing gradient.

4. The method of claim 1 further comprising the step of obtaining said gp140 protein by running said gp140 through affinity chromatography with elution at pH8 followed by a sizing gradient.

5. The method of claim 1, 3 or 4 wherein said gp 140 protein is further defined as missing the gp120/gp41 cleavage site.

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=> e hoxie james a/in
E1           1      HOXIE CAROLINA M/IN
E2           1      HOXIE DEAN M/IN
E3           8 -->  HOXIE JAMES A/IN
E4           1      HOXIE JOHN K/IN
E5           1      HOXIE LINDA M/IN
E6           1      HOXIE PAUL/IN
E7           5      HOXIE STEPHEN W/IN
E8           1      HOXIE VAUGHN C/IN
E9           1      HOXIMEIER RONALD J/IN
E10          1      HOXIT GARY K/IN
E11         40      HOXMEIER RONALD J/IN
E12         21      HOXMEIER RONALD JAMES/IN

=> s e3
L2           8 "HOXIE JAMES A"/IN

=> s l1 not l2
L3           8 L1 NOT L2

=> d l3,ti,1-8
```

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L3   ANSWER 1 OF 8  USPATFULL on STN
TI     ADMINISTRATION OF AN AGENT WHICH DECREASES PROCESSING OF AMYLOID
       PRECURSOR PROTEIN

L3   ANSWER 2 OF 8  USPATFULL on STN
TI     Methods and compositions for modulating the interaction between the APJ
       receptor and the HIV virus

L3   ANSWER 3 OF 8  USPATFULL on STN
TI     Methods and compositions for modulating the interaction between the APJ
       receptor and the HIV virus

L3   ANSWER 4 OF 8  USPATFULL on STN
TI     Methods and compositions for modulating the interaction between the APJ
       receptor and the HIV virus

L3   ANSWER 5 OF 8  USPATFULL on STN
TI     Methods and compositions for modulating the interaction between the APJ
       receptor and the HIV virus

L3   ANSWER 6 OF 8  USPATFULL on STN
TI     METHODS AND COMPOSITIONS FOR MODULATING THE INTERACTION BETWEEN THE APJ
       RECEPTOR AND THE HIV VIRUS

L3   ANSWER 7 OF 8  USPATFULL on STN
TI     Oligomeric HIV-1 envelope glycoproteins

L3   ANSWER 8 OF 8  USPATFULL on STN
TI     Oligomeric HIV-1 envelope glycoproteins


=> del l3
DELETE L3? (Y)/N:y

=> d his

     (FILE 'HOME' ENTERED AT 13:06:04 ON 15 OCT 2006)

     FILE 'USPATFULL' ENTERED AT 13:06:13 ON 15 OCT 2006
                E DOMS ROBERT W/IN
L1           12 S E3
                E HOXIE JAMES A/IN
L2            8 S E3

=> s l2 not l1
L3            4 L2 NOT L1

=> d l3,cbib,clm,1-4

L3   ANSWER 1 OF 4  USPATFULL on STN
2006:82526 Antibodies directed against cellular coreceptors for human
    immunodeficiency virus and methods of using the same.
    Hoxie, James A., Berwyn, PA, UNITED STATES
    The Trustees of the University of Pennsylvania, Philadelphia, PA, UNITED
    STATES (U.S. corporation)
    US 39057 E1 20060404
    US 5994515  19991130 (Original)
    APPLICATION: US 2004-829475  20040422 (10)
    US 1997-882435 19970625 (Original)
    PRIORITY: US 1996-20396P  19960625 (60)
    US 1996-20647P  19960627 (60)
    DOCUMENT TYPE: Reissue; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM    What is claimed is:
       1. An antibody which binds to a cellular chemokine receptor protein
       essential for immunodeficiency virus entry into a cell wherein said
       chemokine receptor protein is ( not CD4 )  CXCR4.

       2. The antibody of claim 1, wherein said immunodeficiency virus is
       selected from the group consisting of HIV-1, HIV-2 and SIV.

       3. The antibody of claim 1, wherein said protein is a cellular protein
       which is an HIV receptor protein.

       4. The antibody of claim 1, wherein said protein is a cellular cofactor
       for a cellular protein which is a HIV receptor protein.

       ( 5. The antibody of claim 4, wherein said protein is selected from the
       group consisting of CXCR4 and CCR5. )

       ( 6. The antibody of claim 5, wherein said protein is CXCR4. )
```

7. The antibody of claim 1, wherein said antibody is selected from the group consisting of a monoclonal antibody and a synthetic antibody.

8. The antibody of claim 7, wherein said antibody is a monoclonal antibody.

9. The antibody of claim 8, wherein said antibody is MAB 12G5.

L3 ANSWER 2 OF 4 USPATFULL on STN
2005:75096 Compositions, methods and kits relating to deletion mutations of immunodeficiency virus gp120 hypervariable regions.
Hoxie, James A., Berwyn, PA, UNITED STATES
Lin, George, Voorhees, NJ, UNITED STATES
The Trustees of the University of Pennylvania (U.S. corporation)
US 2005064392 A1 20050324
APPLICATION: US 2004-767648 A1 20040129 (10)
PRIORITY: US 2003-443364P 20030129 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An isolated nucleic acid encoding a mammalian immunodeficiency virus glycoprotein (gp) 120 polypeptide, or a mutant, derivative, or fragment thereof, wherein said gp120 polypeptide comprises a deletion of hypervariable loop 3 (V3), and further comprises a compensatory mutation.

2. The isolated nucleic acid of claim 1, wherein said mammalian immunodeficiency virus is selected from the group consisting of a simian immunodeficiency virus (SIV), a human immunodeficiency virus type 1 (HIV-1), and a human immunodeficiency virus type 2 (HIV-2).

3. The isolated nucleic acid of claim 2, wherein said mammalian immunodeficiency virus is HIV-2.

4. The isolated nucleic acid of claim 3, wherein said deletion of V3 is selected from the group consisting of a deletion of from about amino acid residue number 303 to amino acid residue number 324 (ΔV3(6,6)) relative to the amino acid sequence of HIV-2/vcp gp120 as provided in SEQ ID NO:5, and a deletion from about amino acid residue number 298 to amino acid residue number 331 (ΔV3(1,1)) relative to the amino acid sequence of HIV-2/vcp gp120 as provided in SEQ ID NO:5.

5. The isolated nucleic acid of claim 3, wherein said deletion of V3 is a deletion from about nucleotide number 894 to nucleotide number 1032 (ΔV3(1,1)) encoding from about amino acid residue number 298 to amino acid residue number 331 relative to the amino acid sequence of HIV-2/vcp gp120 as provided in SEQ ID NO:5.

6. The isolated nucleic acid of claim 4, wherein said gp120 further comprises a deletion of the V1/V2 region.

7. The isolated nucleic acid of claim 1, wherein said compensatory mutation is at least one mutation selected from the group consisting of an amino acid substitution from isoleucine to valine at amino acid residue number 55, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 79, an amino acid substitution from phenylalanine to serine at amino acid residue number 94, an amino acid substitution from aspartic acid to glycine at amino acid residue number 142, an amino acid substitution from threonine to isoleucine at amino acid residue number 160, an amino acid substitution from alanine to threonine at amino acid residue number 173, an amino acid substitution from threonine to lysine at amino acid residue number 202, an amino acid substitution from glutamic acid to lysine at amino acid residue number 203, an amino acid substitution from threonine to isoleucine at amino acid residue number 231, an amino acid substitution from alanine to threonine at amino acid residue number 267, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 279, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 280, an amino acid substitution from glutamic acid to lysine at amino acid residue number 334, an amino acid substitution from glutamic acid to lysine at amino acid residue number 340, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 391, an amino acid substitution from threonine to alanine at amino acid residue number 393, an amino acid substitution from glutamine to arginine at amino acid residue number 399, an amino acid substitution from valine to isoleucine at amino acid residue number 405, an amino acid substitution from valine to isoleucine at amino acid residue number 429, an amino acid substitution from glutamic acid to valine at amino acid residue number 437, an amino acid substitution from threonine to alanine at amino acid residue number 439, and an amino acid substitution from glycine to alanine at amino acid residue number 666, wherein the amino acid residue number of said compensatory mutation is

relative to the amino acid sequence of parental HIV-2/vcp gp120 as provided in SEQ ID NO:5.

8. The isolated nucleic acid of claim 6, wherein said V3 deletion is ΔV3(6,6) and further wherein said compensatory mutation is at least one amino acid substitution selected from the group consisting of an amino acid substitution from isoleucine to valine at amino acid residue number 55, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 79, an amino acid substitution from threonine to lysine at amino acid residue number 202, an amino acid substitution from threonine to isoleucine at amino acid residue number 231, an amino acid substitution from alanine to threonine at amino acid residue number 267, and an amino acid substitution from asparagine to aspartic acid at amino acid residue number 391, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of parental HIV-2/vcp gp120 as provided in SEQ ID NO:5.

9. The isolated nucleic acid of claim 6, wherein said V3 deletion is ΔV3(6,6) and further wherein said compensatory mutation is at least one amino acid substitution selected from the group consisting of an amino acid substitution from isoleucine to valine at amino acid residue number 55, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 79, an amino acid substitution from phenylalanine to serine at amino acid residue number 94, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 280, and an amino acid substitution from asparagine to aspartic acid at amino acid residue number 391, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of parental HIV-2/vcp gp120 as provided in SEQ ID NO:5.

10. The isolated nucleic acid of claim 4, wherein said V3 deletion is ΔV3(6,6) and further wherein said compensatory mutation is at least one amino acid substitution selected from the group consisting of an amino acid substitution from threonine to alanine at amino acid residue number 393, and an amino acid substitution from valine to isoleucine at amino acid residue number 429, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of parental HIV-2/vcp gp120 as provided in SEQ ID NO:5.

11. The isolated nucleic acid of claim 6, wherein said V3 deletion is ΔV3(1,1) and further wherein said compensatory mutation is at least one of an amino acid substitution selected from the group consisting of an amino acid substitution from alanine to threonine at amino acid residue number 173, an amino acid substitution from glutamic acid to lysine at amino acid residue number 203, an amino acid substitution from threonine to alanine at amino acid residue number 393, an amino acid substitution from glutamine to arginine at amino acid residue number 405, an amino acid substitution from valine to isoleucine at amino acid residue number 429, an amino acid substitution from threonine to alanine at amino acid residue number 439, and an amino acid substitution from glycine to alanine at amino acid residue number 666, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of parental HIV-2/vcp gp120 as provided in SEQ ID NO:5.

12. An isolated nucleic acid encoding a mammalian immunodeficiency virus glycoprotein (gp) 120 polypeptide, or a mutant, derivative, or fragment thereof, wherein said gp120 polypeptide comprises a deletion of hypervariable loop 3 (V3), a deletion of hypervariable loops V1/V2, and further comprises a compensatory mutation wherein the nucleic acid sequence of said nucleic acid is selected from ihe group consisting of the sequence of SEQ ID NO:11, the sequence of SEQ ID NO:17, and the sequence of SEQ ID NO:29.

13. The isolated nucleic acid of claim 12, wherein said deletion is selected from the group consisting of a deletion from about amino acid residue number 303 to amino acid residue number 324 (ΔV3(6,6)), and a deletion from about amino acid residue number 298 to amino acid residue number 331 (ΔV3(1,1)), relative to the amino acid sequence of HIV-2/vcp gp120 as provided in SEQ ID NO:5.

14. An isolated nucleic acid encoding a mammalian immunodeficiency virus glycoprotein (gp) 120 polypeptide, or a mutant, derivative, or fragment thereof, wherein said gp120 polypeptide comprises a ΔV3(6,6) deletion, and further comprises a compensatory mutation wherein the nucleic acid sequence of said nucleic acid comprises the sequence of SEQ ID NO:23.

15. The isolated nucleic acid of claim 1, wherein the sequence of said nucleic acid is at least one sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:20, and SEQ ID NO:26.

16. The isolated nucleic acid of claim 1, wherein the amino acid sequence of said gp120 polypeptide encoded by said nucleic acid is selected from the group consisting of the amino acid sequence of SEQ ID NO:11, the amino acid sequence of SEQ ID NO:17, the amino acid sequence of SEQ ID NO:23, and the amino acid sequence of SEQ ID NO:29.

17. An isolated nucleic acid encoding a mammalian immunodeficiency virus gp41 polypeptide, wherein said gp41 polypeptide comprises a compensatory mutation.

18. The isolated nucleic acid of claim 17, wherein the nucleic acid sequence of said isolated nucleic acid is selected from the group consisting of the nucleic acid sequence of SEQ ID NO:9, the sequence of SEQ ID NO:15, the sequence of SEQ ID NO:21, and the sequence of SEQ ID NO:27.

19. The isolated nucleic acid of claim 17, wherein the amino acid sequence of said gp41 polypeptide encoded by said nucleic acid is selected from the group consisting of the amino acid sequence of SEQ ID NO:12, the amino acid sequence of SEQ ID NO:18, the amino acid sequence of SEQ ID NO:24, and the amino acid sequence of SEQ ID NO:30.

20. The isolated nucleic acid of claim 17, wherein said compensatory mutation is a truncation of the cytoplasmic domain.

21. The isolated nucleic acid of claim 17, wherein said compensatory mutation is at least one mutation selected from the group consisting of an amino acid substitution from leucine to valine at amino acid residue number 518, an amino acid substitution from alanine to threonine at amino acid residue number 529, an amino acid substitution from isoleucine to valine at amino acid residue number 531, an amino acid substitution from alanine to threonine at amino acid residue number 561, and an amino acid substitution from alanine to threonine at amino acid residue number 673, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of HIV-2/vcp gp41 (SEQ ID NO:6).

22. The isolated nucleic acid of claim 20, wherein said truncation is selected from the group consisting of a truncation at amino acid residue number 733, a truncation at amino acid residue number 753, a truncation at amino acid residue number 764, wherein the amino acid residue number of said truncation is relative to the amino acid sequence of HIV-2/vcp gp41 (SEQ ID NO:6).

23. An isolated mammalian immunodeficiency virus gp120 polypeptide, wherein said polypeptide comprises a substantial deletion of V3 and further comprises a compensatory mutation.

24. The isolated polypeptide of claim 23, wherein said polypeptide is fusogenic.

25. The isolated gp120 polypeptide of claim 23, wherein said mammalian immunodeficiency virus is selected from the group consisting of a simian immunodeficiency virus (SIV), a human immunodeficiency virus type 1 (HIV-1), and a human immunodeficiency virus type 2 (HIV-2).

26. The isolated gp120 polypeptide of claim 25, wherein said mammalian immunodeficiency virus is HIV-2.

27. The isolated gp120 polypeptide of claim 26, wherein said deletion of V3 is selected from the group consisting of a deletion of from about amino acid residue number 303 to amino acid residue number 324 (ΔV3(6,6)) relative to the amino acid sequence of HIV-2/vcp gp120 as provided in SEQ ID NO:5, and a deletion from about amino acid residue number 298 to amino acid residue number 331 (ΔV3(1,1)) relative to the amino acid sequence of HIV-2/vcp gp120 as provided in SEQ ID NO:5.

28. The isolated gp120 polypeptide of claim 27, wherein said gp120 further comprises a deletion of the V1/V2 region.

29. The isolated gp120 polypeptide of claim 28, wherein said compensatory mutation is at least one mutation selected from the group consisting of an amino acid substitution from isoleucine to valine at amino acid residue number 55, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 79, an amino acid substitution from phenylalanine to serine at amino acid residue number 94, an amino acid substitution from aspartic acid to glycine at amino acid residue number 142, an amino acid substitution from threonine to isoleucine at amino acid residue number 160, an amino acid substitution from alanine to threonine at amino acid residue number 173, an amino acid substitution from threonine to lysine at amino acid residue number 202, an amino acid substitution from glutamic acid to lysine at amino

acid residue number 203, an amino acid substitution from threonine to isoleucine at amino acid residue number 231, an amino acid substitution from alanine to threonine at amino acid residue number 267, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 279, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 280, an amino acid substitution from glutamic acid to lysine at amino acid residue number 334, an amino acid substitution from glutamic acid to lysine at amino acid residue number 340, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 391, an amino acid substitution from threonine to alanine at amino acid residue number 393, an amino acid substitution from valine to isoleucine at amino acid residue number 399, an amino acid substitution from glutamine to arginine at amino acid residue number 405, an amino acid substitution from valine to isoleucine at amino acid residue number 429, an amino acid substitution from glutamic acid to valine at amino acid residue number 437, an amino acid substitution from threonine to alanine at amino acid residue number 439, and an amino acid substitution from glycine to alanine at amino acid residue number 666, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of parental HIV-2/vcp gp120 as provided in SEQ ID NO:5.

30. The isolated gp120 polypeptide of claim 29, wherein said V3 deletion is ΔV3(6,6) and further wherein said compensatory mutation is at least one of an amino acid substitution selected from the group consisting of an amino acid substitution from isoleucine to valine at amino acid residue number 55, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 79, an amino acid substitution from threonine to lysine at amino acid residue number 202, an amino acid substitution from threonine to isoleucine at amino acid residue number 231, an amino acid substitution from alanine to threonine at amino acid residue number 267, and an amino acid substitution from asparagine to aspartic acid at amino acid residue number 391, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of parental HIV-2/vcp gp120 as provided in SEQ ID NO:5.

31. The isolated gp120 polypeptide of claim 29, wherein said V3 deletion is ΔV3(6,6) and further wherein said compensatory mutation is at least one of an amino acid substitution selected from the group consisting of an amino acid substitution from isoleucine to valine at amino acid residue number 55, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 79, an amino acid substitution from phenylalanine to serine at amino acid residue number 94, an amino acid substitution from asparagine to aspartic acid at amino acid residue number 280, and an amino acid substitution from asparagine to aspartic acid at amino acid residue number 391, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of parental HIV-2/vcp gp120 as provided in SEQ ID NO:5.

32. The isolated gp120 polypeptide of claim 27, wherein said V3 deletion is ΔV3(6,6) and further wherein said compensatory mutation is at least one of an amino acid substitution selected from the group consisting of an amino acid substitution from threonine to alanine at amino acid residue number 393, and an amino acid substitution from valine to isoleucine at amino acid residue number 429, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of parental HIV-2/vcp gp120 as provided in SEQ ID NO:5.

33. The isolated gp120 polypeptide of claim 29, wherein said V3 deletion is ΔV3(1,1) and further wherein said compensatory mutation is at least one of an amino acid substitution selected from the group consisting of an amino acid substitution from alanine to threonine at amino acid residue number 173, an amino acid substitution from glutamic acid to lysine at amino acid residue number 203, an amino acid substitution from threonine to alanine at amino acid residue number 393, an amino acid substitution from glutamine to arginine at amino acid residue number 405, an amino acid substitution from valine to isoleucine at amino acid residue number 429, an amino acid substitution from threonine to alanine at amino acid residue number 439, and an amino acid substitution from glycine to alanine at amino acid residue number 666, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of parental HIV-2/vcp gp120 as provided in SEQ ID NO:5.

34. An isolated gp120 polypeptide, or a mutant, derivative, or fragment thereof, wherein said gp120 polypeptide comprises a deletion of hypervariable loop 3 (V3), a deletion of hypervariable loops V1/V2, and further comprises a compensatory mutation wherein the amino acid sequence of said gp120 polypeptide is selected from the group consisting of the sequence of SEQ ID NO:11, the sequence of SEQ ID NO:17, and the

sequence of SEQ ID NO:29.

35. An isolated gp120 polypeptide, or a mutant, derivative, or fragment thereof, wherein said gp120 polypeptide comprises a deletion of hypervariable loop 3 (V3), and further comprises a compensatory mutation wherein the amino acid sequence of said gp120 polypeptide comprises the sequence of SEQ ID NO:23.

36. An isolated mammalian immunodeficiency virus gp41 polypeptide, wherein said gp41 comprises a compensatory mutation.

37. The isolated gp41 polypeptide of claim 36, wherein said compensatory mutation is at least one mutation selected from the group consisting of an amino acid substitution from leucine to valine at amino acid residue number 518, an amino acid substitution from alanine to threonine at amino acid residue number 529, an amino acid substitution from isoleucine to valine at amino acid residue number 531, an amino acid substitution from alanine to threonine at amino acid residue number 561, and an amino acid substitution from alanine to threonine at amino acid residue number 673, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of HIV-2/vcp gp41 (SEQ ID NO:6).

38. The isolated gp41 polypeptide of claim 36, wherein said compensatory mutation is a truncation of the cytoplasmic domain.

39. The isolated gp41 polypeptide of claim 38, wherein said truncation is selected from the group consisting of a truncation at amino acid 733, a truncation at amino acid 753, and a truncation at amino acid 764, wherein the amino acid residue number of said compensatory mutation is relative to the amino acid sequence of HIV-2/vcp gp41 (SEQ ID NO:6).

40. The isolated gp41 polypeptide of claim 36, wherein the amino acid sequence of said polypeptide is selected from the group consisting of the sequence of SEQ ID NO:12, the sequence of SEQ ID NO:18, the sequence of SEQ ID NO:24, and the sequence of SEQ ID NO:30.

41. A composition comprising a mammalian immunodeficiency virus gp120 polypeptide, wherein said gp120 polypeptide comprises a substantial deletion of V3, and a pharmaceutically acceptable carrier.

42. The composition of claim 41, said composition further comprising a mammalian immunodeficiency virus gp41 polypeptide, wherein said gp41 comprises a compensatory mutation.

43. The composition of claim 42, wherein said gp120 further comprises a deletion of V1/V2.

44. The composition of claim 43, wherein the amino acid sequence of said gp120 polypeptide comprises at least one sequence selected from the group consisting of the sequence of the sequence of SEQ ID NO:11, the sequence of SEQ ID NO:17, and the sequence of SEQ ID NO:29.

45. The composition of claim 44, wherein the amino acid sequence of said gp41 polypeptide comprises at least one sequence selected from the group consisting of the sequence of the sequence of SEQ ID NO:12, the sequence of SEQ ID NO:18, and the sequence of SEQ ID NO:30.

46. The composition of claim 42, wherein the amino acid sequence of said gp120 polypeptide comprises at least one sequence selected from the group consisting of the sequence of the sequence of SEQ ID NO:11, the sequence of SEQ ID NO:17, the sequence of SEQ ID NO:23, and the sequence of SEQ ID NO:29.

47. The composition of claim 46, wherein the amino acid sequence of said gp41 polypeptide comprises at least one sequence selected from the group consisting of the sequence of the sequence of SEQ ID NO:12, the sequence of SEQ ID NO:18, the sequence of SEQ ID NO:24, and the sequence of SEQ ID NO:30.

48. An isolated mammalian immunodeficiency virus, said virus comprising a gp120 polypeptide wherein said gp120 comprises a substantial deletion of V3.

49. The isolated virus of claim 48, wherein said virus is fusion-competent.

50. The isolated virus of claim 49, wherein said virus is replication-competent.

51. The isolated virus of claim 50, said virus further comprising a gp41 polypeptide wherein said gp41 comprises a compensatory mutation.

52. The isolated virus of claim [illegible], wherein said gp[illegible] polypeptide comprises a compensatory mutation.

53. The isolated virus of claim 52, wherein the amino acid sequence of said gp120 polypeptide comprises at least one sequence selected from the group consisting of the sequence of the sequence of SEQ ID NO:11, the sequence of SEQ ID NO:17, the sequence of SEQ ID NO:23, and the sequence of SEQ ID NO:29.

54. The isolated virus of claim 53, wherein the amino acid sequence of said gp41 polypeptide comprises at least one sequence selected from the group consisting of the sequence of the sequence of SEQ ID NO:12, the sequence of SEQ ID NO:18, the sequence of SEQ ID NO:24, and the sequence of SEQ ID NO:30.

55. An isolated mammalian immunodeficiency virus Env, wherein said Env comprises a substantial deletion of V3 and further wherein said Env is fusogenic.

56. The isolated mammalian immunodeficiency virus Env of claim 55, wherein the amino acid sequence of said Env comprises at least one sequence selected from the group consisting of the sequence of SEQ ID NO:10, the sequence of SEQ ID NO:16, the sequence of SEQ ID NO:22, and the sequence of SEQ ID NO:28.

57. A method of producing a neutralizing antibody in a mammal in need thereof, said method comprising administering to a mammal an immunogenic amount of an isolated gp120, wherein said gp120 comprises a substantial deletion of V3, and further comprises a deletion of V1/V2, thereby producing said neutralizing antibody in said mammal.

58. The method of claim 57, wherein the amino acid sequence of said isolated gp120 comprises at least one sequence selected from the group consisting of the sequence of SEQ ID NO:11, the sequence of SEQ ID NO:17, the sequence of SEQ ID NO:23, and the sequence of SEQ ID NO:29.

59. The method of claim 57, wherein said gp120 further comprises a deletion of V4.

60. A method of eliciting a neutralizing antibody in a mammal, said method comprising administering an immunogenic amount of the composition of claim 43, thereby eliciting said neutralizing antibody in said mammal.

61. The method of claim 60, wherein said mammal is selected from the group consisting of an ape, and a human.

62. An antibody produced by the method of claim 60.

63. An antibody elicited by the method of claim 57.

64. A method of producing a replication-competent mammalian immunodeficiency virus comprising a deletion of at least one hypervariable loop domain, said method comprising a) producing a virus comprising gp120 wherein said gp120 comprises a deletion of V1/V2, said gp120 further comprising a substantial deletion of V3; b) passaging said virus in cell culture and selecting for a virus that is capable of fusing with a cell; c) introducing into said virus selected in (b) a gp41 comprising enhanced fusogenecity wherein said gp41 comprises at least one compensatory mutation; and d) passaging the virus of (c) in cell culture and selecting for a virus that is capable of fusing with a cell; thereby producing said replication-competent virus.

65. A replication-competent virus produced by the method of claim 64.

66. A method of identifying a determinant of a chemokine receptor that specifically binds with a gp120 polypeptide of a mammalian immunodeficiency virus, said method comprising contacting a high-affinity gp120 polypeptide of said virus with a panel of mutants of said chemokine receptor, assessing the binding of said gp120 polypeptide with each of said mutants, and comparing said binding of said gp120 with each of said mutants, thereby identifying said determinant of said chemokine receptor that specifically binds with said gp120.

67. A method of identifying a compound that inhibits binding of a mammalian immunodeficiency virus gp120 polypeptide with a chemokine receptor, said method comprising assessing the level of binding of a gp120 polypeptide comprising a deletion of V1/V2, and a substantial deletion of V3, wherein said gp120 is fusogenic, with a chemokine receptor in the presence of a compound, and comparing the level of binding of said gp120 with said chemokine receptor in the presence of said compound with the binding of an otherwise identical gp120 with an otherwise identical chemokine receptor in the absence of said compound,

wherein a lower level of binding of said gp120 with said chemokine receptor in the presence of said compound compared with the level of binding of said otherwise identical gp120 with said otherwise identical chemokine receptor in the absence of said compound is an indication that said compound inhibits binding of said gp120 with said chemokine receptor, thereby identifying a compound that inhibits binding of said gp120 with said chemokine receptor.

68. A kit for producing an immunodeficiency virus-neutralizing antibody in a mammal, said kit comprising an immunogenic amount of a gp120 polypeptide of said mammalian immunodeficiency virus, wherein said gp120 comprises a deletion of V1/V2, and a substantial deletion of V3, said kit further comprising an applicator, and an instructional material for the use thereof.

69. The kit of claim 68, wherein the amino acid sequence of said gp120 polypeptide is at least one sequence selected from the group consisting of group consisting of the sequence of SEQ ID NO:11, the sequence of SEQ ID NO:17, the sequence of SEQ ID NO:23, and the sequence of SEQ ID NO:29.

70. A kit for producing an immunodeficiency virus-neutralizing antibody in a mammal, said kit comprising an immunogenic amount of a mammalian immunodeficiency virus Env, wherein said Env comprises a deletion of V1/V2, and a substantial deletion of V3, and further wherein said Env comprises a compensatory mutation, said kit further comprising an applicator, and an instructional material for the use thereof.

71. The kit of claim 70, wherein the amino acid sequence of said Env comprises at least one sequence selected from the group consisting of the sequence of SEQ ID NO:10, the sequence of SEQ ID NO:16, the sequence of SEQ ID NO:22, and the sequence of SEQ ID NO:28.

72. A kit for eliciting a neutralizing antibody in a mammal, said kit comprising an immunogenic amount of the composition of claim 43, said kit further comprising an applicator, and an instructional material for the use thereof.

L3 ANSWER 3 OF 4 USPATFULL on STN
1999:155898 Antibodies directed against cellular coreceptors for human immunodeficiency virus and methods of using the same.
Hoxie, James A., Berwyn, PA, United States
Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 5994515 19991130
APPLICATION: US 1997-882435 19970625 (8)
PRIORITY: US 1996-20396P 19960627 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An antibody which binds to a cellular chemokine receptor protein essential for immunodeficiency virus entry into a cell wherein said chemokine receptor protein is not CD4.

2. The antibody of claim 1, wherein said immunodeficiency virus is selected from the group consisting of HIV-1, HIV-2 and SIV.

3. The antibody of claim 1, wherein said protein is a cellular protein which is an HIV receptor protein.

4. The antibody of claim 1, wherein said protein is a cellular cofactor for a cellular protein which is a HIV receptor protein.

5. The antibody of claim 4, wherein said protein is selected from the group consisting of CXCR4 and CCR5.

6. The antibody of claim 5, wherein said protein is CXCR4.

7. The antibody of claim 1, wherein said antibody is selected from the group consisting of a monoclonal antibody and a synthetic antibody.

8. The antibody of claim 7, wherein said antibody is a monoclonal antibody.

9. The antibody of claim 8, wherein said antibody is MAB 12G5.

L3 ANSWER 4 OF 4 USPATFULL on STN
1998:147222 Method and kit for detection of thrombin receptor activation of platelets and other cells.
Brass, Lawrence, Bala Cynwyd, PA, United States
Hoxie, James A., Berwyn, PA, United States

Trustees of the University of Pennsylvania, Philadelphia, PA, United States
(U.S. corporation)
US 5840499 19981124
APPLICATION: US 1996-659486 19960606 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method of diagnosing a pre-thrombotic state in a patient comprising: (a) obtaining a sample comprising whole blood, serum, plasma or urine from a patient; (b) contacting a solid phase support coated with a peptide consisting of SEQ ID NO: 2 with the sample and a known amount of an antibody which specifically binds to the peptide consisting of SEQ ID NO: 2 so that the peptide consisting of SEQ ID NO: 2 and any peptide fragment comprising the N-terminal fragment of the thrombin receptor cleaved by thrombin between residues Ser^{42} and Arg^{41} in the sample can specifically bind to the antibody; (c) detecting any antibody bound specifically to the peptide consisting of SEQ ID NO: 2 on the solid phase support; and (d) quantifying the amount of peptide comprising the N-terminal fragment of the thrombin receptor cleaved by thrombin between residues Ser^{42} and Arg^{41} in the sample based on an amount of specifically bound antibody detected so that a pre-thrombotic state in the patient can be diagnosed.

2. A kit useful for detecting thrombin-induced cell activation and diagnosing a pre-thrombotic state in a patient comprising: (a) a solid phase support; (b) a peptide consisting of SEQ ID NO: 2; and (c) a monoclonal antibody which specifically binds to the peptide consisting of SEQ ID.NO: 2.

3. A method of detecting thrombin-induced cell activation in a subject comprising: (a) obtaining a sample of whole blood, serum, plasma or urine from the subject; (b) contacting a solid phase support which has been coated with a peptide consisting of SEQ ID NO: 2 with the sample and a known amount of an antibody which specifically binds to the peptide consisting of SEQ ID NO: 2; and (c) detecting any antibody specifically bound to the peptide consisting of SEQ ID NO: 2 on the solid phase support so that the presence or absence of a peptide comprising the N-terminal fragment of a thrombin receptor cleaved by thrombin between residues Ser^{42} and Arg^{41} which is indicative of thrombin-induced cell activation can be determined.

```
=> s bates paul/in
L4            3 BATES PAUL/IN

=> del l4
DELETE L4? (Y)/N:y

=> e bates paul/in
E1            1      BATES PATRICIA ANN/IN
E2            1      BATES PATTI L/IN
E3            3 --> BATES PAUL/IN
E4            1      BATES PAUL A/IN
E5            1      BATES PAUL E/IN
E6            1      BATES PAUL EDWARD/IN
E7            2      BATES PAUL V/IN
E8            4      BATES PAULA J/IN
E9            2      BATES PERRY C/IN
E10           2      BATES PERSONAL REPRESENTATIVE BY H RICHARD/IN
E11           1      BATES PERSONAL REPRESENTATIVE H RICHARD/IN
E12           1      BATES PETER/IN

=> s e3-e6
              3 "BATES PAUL"/IN
              1 "BATES PAUL A"/IN
              1 "BATES PAUL E"/IN
              1 "BATES PAUL EDWARD"/IN
L4            6 ("BATES PAUL"/IN OR "BATES PAUL A"/IN OR "BATES PAUL E"/IN OR
                "BATES PAUL EDWARD"/IN)

=> s l4 not (l1 or l2)
L5            5 L4 NOT (L1 OR L2)

=> d l5,ti,1-5

L5   ANSWER 1 OF 5  USPATFULL on STN
TI     Resilient cushioning device for the heel portion of a sole

L5   ANSWER 2 OF 5  USPATFULL on STN
TI     Receptor-binding pocket mutants of influenza a virus hemagglutinin for
       use in targeted gene delivery
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L5   ANSWER 3 OF 5  USPATFULL on STN
TI     Monoclonal antibodies for use in diagnosis and treatment of colorectal
       cancer

L5   ANSWER 4 OF 5  USPATFULL on STN
TI     Process for making lyophilized radiographic imaging kit

L5   ANSWER 5 OF 5  USPATFULL on STN
TI     Preparation of French fries


=> e hoffman trevor l/in
E1            1      HOFFMAN TONY/IN
E2            1      HOFFMAN TORSTEN/IN
E3            4 --> HOFFMAN TREVOR L/IN
E4            1      HOFFMAN UDO/IN
E5            1      HOFFMAN ULRICH/IN
E6            1      HOFFMAN VALERIE L/IN
E7            2      HOFFMAN VANCE E/IN
E8            1      HOFFMAN VASILIOS F/IN
E9            1      HOFFMAN VICTOR L/IN
E10           1      HOFFMAN VIKTORIA/IN
E11           2      HOFFMAN VINCENT L/IN
E12           2      HOFFMAN VIRGINIA M/IN

=> s e3
L6            4 "HOFFMAN TREVOR L"/IN

=> s 16 not (11 or 12)
L7            0 L6 NOT (L1 OR L2)

=> e rucker joseph/in
E1            2      RUCKER JON A/IN
E2            2      RUCKER JON S JR/IN
E3            1 --> RUCKER JOSEPH/IN
E4            2      RUCKER JOSEPH R/IN
E5            1      RUCKER JR JOSEPH W/IN
E6            1      RUCKER JULIA/IN
E7            1      RUCKER JULIA A/IN
E8            5      RUCKER KENNETH S/IN
E9            2      RUCKER KEVIN S/IN
E10           1      RUCKER KEVIN SCOTT/IN
E11           5      RUCKER KLAUS G/IN
E12           1      RUCKER LANCE M/IN

=> s e3-e5
              1 "RUCKER JOSEPH"/IN
              2 "RUCKER JOSEPH R"/IN
              1 "RUCKER JR JOSEPH W"/IN
L8            4 ("RUCKER JOSEPH"/IN OR "RUCKER JOSEPH R"/IN OR "RUCKER JR JOSEPH
                W"/IN)

=> s 18 not (11 or 12)
L9            3 L8 NOT (L1 OR L2)

=> d 19,ti,1-3

L9   ANSWER 1 OF 3  USPATFULL on STN
TI     Gas delivery tube

L9   ANSWER 2 OF 3  USPATFULL on STN
TI     Speaker mounting system

L9   ANSWER 3 OF 3  USPATFULL on STN
TI     Speaker mounting system


=> file wpids
COST IN U.S. DOLLARS                               SINCE FILE       TOTAL
                                                        ENTRY     SESSION
FULL ESTIMATED COST                                     42.01       42.22

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    INDEX ENHANCEMENTS PLEASE VISIT:
http://www.stn-international.de/stndatabases/details/dwpi_r.html <<<

=> e doms robert w/in
E1             2      DOMS R/IN
E2             6      DOMS R W/IN
E3             0 --> DOMS ROBERT W/IN
E4             1      DOMS T/IN
E5             1      DOMSA A/IN
E6             1      DOMSA F/IN
E7             6      DOMSA K/IN
E8             1      DOMSA O/IN
E9             1      DOMSA S B/IN
E10            1      DOMSALLA E/IN
E11            4      DOMSCH A/IN
E12           11      DOMSCH H/IN

=> s e1-e2
               2 "DOMS R"/IN
               6 "DOMS R W"/IN
L10            7 ("DOMS R"/IN OR "DOMS R W"/IN)

=> d l10,bib,ab,1-7

L10  ANSWER 1 OF 7  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN   2003-328633 [31]    WPIDS
DNN  N2003-262818        DNC C2003-085468
TI   Isolated lipoparticle for delivering composition to target cells, contains
     membrane spanning proteins.
DC   B04 D16 S03
IN   BATES, P; DOMS, R W; HOFFMAN, T L; HOXIE, J A; RUCKER, J
PA   (UYPE-N) UNIV PENNSYLVANIA
CYC  1
PI   US 2002183247   A1 20021205 (200331)*      45
ADT  US 2002183247 A1 Provisional US 1997-47226P 19970520, CIP of US 1998-6678
     19980113, Provisional US 2000-257988P 20001222, US 2001-32311 20011221
PRAI US 2001-32311          20011221; US 1997-47226P       19970520;
     US 1998-6678           19980113; US 2000-257988P      20001222
AB   US2002183247 A UPAB: 20030516
     NOVELTY - An isolated lipoparticle comprising membrane spanning proteins,
     is new. The protein is not CD63.
          DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:
          (1) a composition comprises the isolated lipoparticle attached to a
     sensor surface;
          (2) assessing the binding interaction of a membrane spanning protein
     with a ligand, comprising:
          (a) producing the lipoparticle;
          (b) attaching the lipoparticle to a substrate;
          (c) contacting the protein present on the lipoparticle with a ligand
     of the protein; and
          (d) detecting any change in the substrate compared with any change in
     an otherwise identical substrate where the protein present on the
     lipoparticle is not contacted with the ligand; and
          (3) a kit for assessing the binding interaction of the membrane
     spanning protein with the ligand, comprising:
          (a) the lipoparticle;
          (b) a substrate;
          (c) an applicator; and
          (d) an instructional material.
          The detecting step assesses the binding interaction of the protein
     with the ligand.
          USE - The lipoparticle is used to deliver a composition to a target
     cell. It can be used to assess the binding of the protein presented in a
     lipid bilayer of the particle with a test component and/or to assess the
     effect of a test compound on the binding of the protein with a cognate
     ligand. It can be used to produce a cellular virus receptor protein to
     obtain quantities of the protein which can be easily purified, relative to
     purification of the protein from the physiological cellular source of the
     protein.
          ADVANTAGE - The inventive lipoparticle is capable of binding to and
     fusing with a target cell which comprises a viral envelope protein to
     which the cellular virus receptor protein is cognate. It allows
     presentation of a cell membrane protein while preserving its biological
     function, so that the interaction of the protein with its cognate ligand
```

complex membrane proteins within a particulate format that is suitable for gene delivery, microfluidics, biosensors, and antigen presentation. It does not encompass cell membrane vesicles, which are typically produced using empirical methods and which are usually heterogeneous in size. It is, preferably, dense, spherical and/or homogeneous in size. The membrane protein in the lipoparticle maintains its structure compared with the native protein when present in the membrane.
Dwg.12/12

L10 ANSWER 2 OF 7 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2001-167730 [17] WPIDS
CR 2000-270121 [23]
DNC C2001-049946
TI Immunogenic composition for stimulating mammalian immune response, comprises recombinant uncleaved gp140 protein retaining its oligomeric structure such that antibodies against HIV envelope proteins are produced.
DC B04
IN BRODER, C C; **DOMS, R W**; EARL, P L; MOSS, B
PA (USSH) US DEPT HEALTH & HUMAN SERVICES
CYC 1
PI US 6171596 B1 20010109 (200117)* 24
ADT US 6171596 B1 Div ex US 1993-165314 19931210, CIP of US 1997-805889 19970303, US 1998-70291 19980430
FDT US 6171596 B1 CIP of US 6039957
PRAI US 1998-70291 19980430; US 1993-165314 19931210;
US 1997-805889 19970303
AB US 6171596 B UPAB: 20010328
NOVELTY - An immunogenic composition (I) comprising a recombinant uncleaved gp140 protein (II) which is a C-terminally truncated form of HIV-1 gp160 protein, missing the gp41 transmembrane domain, and retaining its oligomeric structure, such that neutralizing antibodies against conformational epitopes of HIV-1 envelope proteins found on the oligomeric structure of (II) are produced in an immunized human, is new.
ACTIVITY - AntiHIV.
MECHANISM OF ACTION - Vaccine. Four macaques were immunized with oligomeric gp140 while two macaques served as unimmunized controls. The immunized macaques were each administered with 500 mu l of a composition that included 300 mu g of purified oligomeric gp 140 dispersed in PBS and QS21 adjuvant, intramuscularly at 0, 4, 8 and 24 week time points for a total of four administrations. Chimeric simian immuno virus (SIV) viruses were tested in in vitro neutralization assays to verify that neutralizing antibodies had been produced before proceeding with the virus challenge. Results indicated that the immunogenic composition which included oligomeric gp140 stimulated a broadly reactive immune response that inhibited subsequent virus challenge. The results clearly showed that animals administered with oligomeric gap140 had high neutralizing antibody titers against SHIV-HXB2 after the third and fourth immunizations at 2 and 6 months, respectively.
USE - (I) is useful for stimulating an anti-HIV-1 env immune response in a mammal, by stimulating the formation of neutralizing antibodies against conformational epitopes of HIV-1 env protein in a mammal. gp140 is also useful for preventing HIV infection in a mammal.
Dwg.0/1

L10 ANSWER 3 OF 7 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2001-025132 [03] WPIDS
DNC C2001-007745
TI Novel nucleic acid encoding a CD4-independent human immunodeficiency virus-1 env or its mutant, useful as vaccine for treating HIV-1 infection in humans.
DC B04 D16
IN **DOMS, R W**; HOFFMAN, T L; HOXIE, J A; LABRANCHE, C C
PA (UYPE-N) UNIV PENNSYLVANIA; (DOMS-I) DOMS R W; (HOFF-I) HOFFMAN T L; (HOXI-I) HOXIE J A; (LABR-I) LABRANCHE C C; (UYDU-N) UNIV DUKE
CYC 23
PI WO 2000071561 A1 20001130 (200103)* EN 120
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA JP
AU 2000051378 A 20001212 (200115)
EP 1185545 A1 20020313 (200225) EN
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
US 6420545 B1 20020716 (200248)
US 2003091594 A1 20030515 (200335)
JP 2004522401 W 20040729 (200452) 325
US 6849261 B2 20050201 (200511)
US 2005191621 A1 20050901 (200558)
ADT WO 2000071561 A1 WO 2000-US13487 20000516; AU 2000051378 A AU 2000-51378 20000516; EP 1185545 A1 EP 2000-936004 20000516, WO 2000-US13487 20000516; US 6420545 B1 CIP of US 1999-317556 19990524, US 1999-337387 19990622; US 2003091594 A1 CIP of US 1999-317556 19990524, Div ex US 1999-337387

20000516, WO 2000-US13487 20000516; US 6849261 B2 CIP of US 1999-317556 19990524, Div ex US 1999-337387 19990622, US 2002-196515 20020716; US 2005191621 A1 CIP of US 1999-317556 19990524, Div ex US 1999-337387 19990622, Div ex US 2002-196515 20020716, US 2005-48554 20050201

FDT AU 2000051378 A Based on WO 2000071561; EP 1185545 A1 Based on WO 2000071561; US 2003091594 A1 Div ex US 6420545; JP 2004522401 W Based on WO 2000071561; US 6849261 B2 Div ex US 6420545; US 2005191621 A1 Div ex US 6420545, Div ex US 6849261

PRAI US 1999-337387 19990622; US 1999-317556 19990524;
US 2002-196515 20020716; US 2005-48554 20050201

AB WO 200071561 A UPAB: 20010116

NOVELTY - An isolated nucleic acid sequence (I) encoding a CD4-independent human immunodeficiency virus-1 (HIV-1) env, or its mutant, derivative or fragment, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated nucleic acid (Ia) comprising a portion of HIV-1 env gene which confers CD4 independence on at least one HIV-1 env clone;

(2) a chimeric nucleic acid (II) comprising a first portion encoding at least a portion of HIV-1/IIIBx 8x env coding sequence and a second portion encoding at least a portion of HIV-1 env coding sequence which is not 8x env;

(3) an isolated HIV-1 gp120 polypeptide (III) comprising a stably exposed chemokine coreceptor binding site (CRBS);

(4) an isolated polypeptide (IIIa) comprising HIV-1/IIIBx 8x Env;

(5) a chimeric HIV-1 Env polypeptide (IV) comprising a gp120 polypeptide, and further comprising a first portion comprising HIV-1/IIIBx 8x gp120, and a second portion comprising a gp120 from an HIV-1 other than HIV-1/IIIBx 8x;

(6) a composition (C) comprising a CD4-independent HIV-1 Env comprising (III), where HIV-1 is more sensitive to antibody neutralization than an otherwise identical HIV-1 which does not comprise a stably exposed CRBS;

(7) a pharmaceutical composition (PC) comprising CD4-independent HIV-1 Env protein comprising at least one mutation causing the CRBS to be stably exposed;

(8) a vaccine (V) comprising CD4-independent HIV-1 Env;

(9) a vector (VI) comprising (I), (Ia), or (II);

(10) a host cell (VII) comprising (I), (Ia), (II), (III), (IIIa), (IV) or (C);

(11) identifying an amino acid residue of a HIV-1 Env protein which is involved in CD4 independence, by obtaining a full length env coding sequence from an Env clone which is CD4-independent, and replacing at least a portion of the env coding sequence with a coding sequence from an Env clone which is CD4-dependent to form a chimera, so that when the chimera is CD4-dependent, it is an indication that the portion of env coding sequence is involved in CD4-independence, thus identifying the amino acid residue involved in CD4-independence;

(12) identifying a compound which affects exposure of an HIV-1 gp120 CRBS, by contacting a cell with the compound prior to or contemporaneous with contacting the cell with a labeled gp120 with or without pre-incubation of the gp120 with soluble CD4, measuring the amount of label bound to the cell, and comparing the amount of label bound to the cell contacted with the compound to the amount of label bound to an otherwise identical cell not contacted with the compound, such that a higher or lower amount of label bound to the cell contacted with the compound than the control, indicates that the compound affects exposure of HIV-1 gp120 CRBS;

(13) identifying (M1) a small molecule which inhibits binding of an HIV-1 gp120 using its CRBS to a chemokine receptor, by contacting a cell with the molecule prior to or contemporaneous with contacting the cell with labeled gp120 with or without preincubation of gp120 with soluble CD4, measuring the amount of label bound to the cell, and comparing the amount of label bound to the cell contacted with the molecule with the amount of label bound to an otherwise identical cell not contacted with a molecule, so that a lower amount of label bound to the cell contacted with the molecule compared to the control, is an indication that the molecule inhibits binding of an HIV-1 gp120 using its CRBS to a chemokine receptor;

(14) producing a CD4-independent chimeric HIV-1 Env clone comprising a variable CRBS, by replacing the hypervariable V3-loop of the CD4-independent Env clone with the V3 loop of another HIV-1 comprising a different CRBS than that of CD4-independent Env clone; and

(15) a composition comprising a CD4-independent HIV-1 Env and at least one compound for treating HIV infection.

ACTIVITY - Anti-HIV; antiviral.

MECHANISM OF ACTION - Vaccine. No supporting data given.

USE - (M1) is useful for identifying a small molecule which is useful for inhibiting HIV-1 gp120 binding using its CRBS to a chemokine receptor, and for inhibiting HIV-1 infection of a cell.. CD4-independent HIV-1 Env protein is useful for eliciting an immune response to an HIV-1 chemokine receptor binding site in a mammal. The composition comprising CD4-independent HIV-1 Env protein is useful for treating HIV-1 infection

Dwg.0/14

L10 ANSWER 4 OF 7 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2000-270121 [23] WPIDS
CR 2001-167730 [10]
DNC C2000-082366
TI Producing neutralizing antibodies useful for preventing, treating and diagnosing an HIV infection in a mammal comprises administering recombinant uncleaved gp140 proteins to a human.
DC B04 D16
IN BRODER, C C; **DOMS, R W**; EARL, P L; MOSS, B
PA (USSH) US DEPT HEALTH & HUMAN SERVICES
CYC 1
PI US 6039957 A 20000321 (200023)* 15
ADT US 6039957 A Div ex US 1993-165314 19931210, US 1997-805889 19970303
PRAI US 1993-165314 19931210; US 1997-805889 19970303
AB US 6039957 A UPAB: 20010328
NOVELTY - A method for producing neutralizing antibodies (I) against conformational epitopes of human immunodeficiency virus-1 (HIV-1) envelope proteins in a human, is new.
DETAILED DESCRIPTION - The method comprises administering, to a human, a recombinant uncleaved gp140 proteins retaining its oligomeric structures. The human will produces (I) against conformational epitopes of HIV-1 envelope proteins found on the oligomeric structure of the gp140. gp140 protein is a C-terminally truncated form of HIV-1 gp160 protein, lacking the gp41 transmembrane domain.
ACTIVITY - Anti-HIV. 1-10,000 mu g/kg of anti-gp140 are injected into patients. A (control) patient, suffering from HIV infection was injected with an antibody with specificity for a non-HIV epitope. After 1 week, progress of the HIV infection was measured. The control patient had an increased progression of HIV infection as compared to the patient injected with the anti-gp140 antibodies.
MECHANISM OF ACTION - Envelope (ENP)-CD4 binding blocker.
USE - gp140 is useful for preventing and diagnosing an HIV infection in a mammal. Antibodies against gp140 are useful for treating an HIV infection. The diagnostic method involves isolating body fluid, preferably blood, and contacting it with a labeled monoclonal antibody for gp140. Binding of the antibody to HIV epitopes is detected, using e.g. enzyme linked immunosorbant assay, if the label is an enzyme, radioactive, or fluorescent.
ADVANTAGE - Protein helps in stimulating production of broadly neutralizing antibodies against various strains of HIV-1.
Dwg.0/1

L10 ANSWER 5 OF 7 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2000-256972 [22] WPIDS
DNN N2000-191021 DNC C2000-078541
TI New recombinant eukaryotic cells coexpressing APJ and CD4 polypeptides, useful for identifying compounds that modulate interaction between an HIV virus and an APJ receptor e.g. to develop anti-HIV drugs.
DC B04 D16 S03
IN **DOMS, R**; FAULDS, D; HESSELGESSER, J E; HORUK, R; MITROVIC, B; ZHOU, Y; **DOMS, R W**
PA (SCHD) SCHERING AG; (DOMS-I) DOMS R W; (FAUL-I) FAULDS D; (HESS-I) HESSELGESSER J E; (HORU-I) HORUK R; (MITR-I) MITROVIC B; (ZHOU-I) ZHOU Y; (UYPE-N) UNIV PENNSYLVANIA
CYC 89
PI WO 2000014220 A1 20000316 (200022)* EN 67
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW
AU 9959737 A 20000327 (200032)
NO 2001001162 A 20010307 (200131)
BR 9913635 A 20010522 (200132)
EP 1109906 A1 20010627 (200137) EN
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
CZ 2001000846 A3 20010711 (200147)
SK 2001000308 A3 20010911 (200159)
HU 2001003768 A2 20020228 (200223)
MX 2001000473 A1 20010701 (200236)
US 2002062488 A1 20020523 (200239)
KR 2002013467 A 20020220 (200257)
US 6475718 B2 20021105 (200276)
JP 2002532061 W 20021002 (200279) 70
US 2003003447 A1 20030102 (200305)
US 2003008279 A1 20030109 (200311)
US 2003008376 A1 20030109 (200311)

AU 767484 B 20031113 (200381)
RU 2225721 C2 20040320 (200428)

ADT WO 2000014220 A1 WO 1999-EP6553 19990907; AU 9959737 A AU 1999-59737 19990907; NO 2001001162 A WO 1999-EP6553 19990907, NO 2001-1162 20010307; BR 9913635 A BR 1999-13635 19990907, WO 1999-EP6553 19990907; EP 1109906 A1 EP 1999-968679 19990907, WO 1999-EP6553 19990907; CZ 2001000846 A3 WO 1999-EP6553 19990907, CZ 2001-846 19990907; SK 2001000308 A3 WO 1999-EP6553 19990907, SK 2001-308 19990907; HU 2001003768 A2 WO 1999-EP6553 19990907, HU 2001-3768 19990907; MX 2001000473 A1 MX 2001-473 20010115; US 2002062488 A1 US 1998-149045 19980908; KR 2002013467 A KR 2001-702905 20010306; US 6475718 B2 US 1998-149045 19980908; JP 2002532061 W WO 1999-EP6553 19990907, JP 2000-568964 19990907; US 2003003447 A1 Div ex US 1998-149045 19980908, US 2002-166359 20020611; US 2003008279 A1 Div ex US 1998-149045 19980908, US 2002-166113 20020611; US 2003008376 A1 Div ex US 1998-149045 19980908, US 2002-166357 20020611; US 2003008391 A1 Div ex US 1998-149045 19980908, US 2002-166372 20020611; AU 767484 B AU 1999-59737 19990907; RU 2225721 C2 WO 1999-EP6553 19990907, RU 2001-109234 19990907

FDT AU 9959737 A Based on WO 2000014220; BR 9913635 A Based on WO 2000014220; EP 1109906 A1 Based on WO 2000014220; CZ 2001000846 A3 Based on WO 2000014220; SK 2001000308 A3 Based on WO 2000014220; HU 2001003768 A2 Based on WO 2000014220; JP 2002532061 W Based on WO 2000014220; US 2003003447 A1 Div ex US 6475718; US 2003008279 A1 Div ex US 6475718; US 2003008376 A1 Div ex US 6475718; US 2003008391 A1 Div ex US 6475718; AU 767484 B Previous Publ. AU 9959737, Based on WO 2000014220; RU 2225721 C2 Based on WO 2000014220

PRAI US 1998-149045 19980908; US 2002-166359 20020611;
US 2002-166113 20020611; US 2002-166357 20020611;
US 2002-166372 20020611

AB WO 200014220 A UPAB: 20000508

NOVELTY - Recombinant eukaryotic cells transformed with either one or both of a polynucleotide encoding an APJ polypeptide and a polynucleotide encoding CD4 polypeptide, such that the cell coexpresses APJ and CD4 polypeptides, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) antibodies specifically binding to an extracellular domain of APJ. These inhibit membrane fusion between a cell coexpressing APJ and CD4 polypeptides (I) and a second cell expressing an HIV envelope (env) protein (II), thereby inhibiting HIV infection of (I);

(2) purified peptide fragments of APJ which inhibit cell fusion between (I) and (II), thereby inhibiting HIV infection of (I);

(3) identifying compounds that modulate the interaction between an HIV virus and an APJ receptor comprising:

(a) incubating (I) with (II) under conditions promoting cell fusion with/without a test compound, and determining whether cell fusion between the two cell lines is inhibited by the compound; or

(b) incubating (I) with a test virus carrying an env protein in the presence/absence of a test compound and determining (e.g. as in (a)) whether the compound inhibits cell infection by the virus.

(4) inhibiting HIV infection of cells expressing APJ and CD4 polypeptides by contacting with an APJ binding or blocking agent; and

(5) transgenic non-human animals comprising a phenotype characterized by expression of APJ and CD4 polypeptides not naturally occurring in the animal, conferred by a transgene encoding an APJ polypeptide and a CD4 polypeptide contained in the somatic and germ cells.

USE - The cells can be used to identify compounds that modulate interaction between an HIV virus and an APJ receptor (claimed), which may be useful in the development of anti-HIV drugs, since APJ was shown to function as a coreceptor for cellular infection by several strains of the HIV-1 virus. Contacting cells expressing APJ and CD4 polypeptides with an APJ binding/blocking agent (e.g. the antibodies or peptides) in the method of (3) is useful to inhibit HIV infection of the cells, e.g. to treat subjects having an HIV-related disorder associated with APJ expression (claimed). The antibodies and peptide fragments can be included in medicines and administered to treat subjects (especially fetuses (claimed)) having, or at risk of developing, an HIV infection or related disorder (claimed). The antibodies can also be used detect cells expressing the APJ receptor, useful to diagnose susceptibility to HIV infection (e.g. higher levels in central nervous system tissues may indicate an increased risk of neuropathogenesis associated with HIV infection). The transgenic animals of (4) provide model systems for studying HIV infection and anti-HIV drug development.

ADVANTAGE - The new recombinant cells express additional coreceptors for the HIV virus e.g. APJ polypeptide to CD4 receptors and so provide an important tool for investigating and controlling HIV infection.

Dwg.0/8

Full Text
AN 1999-371466 [31] WPIDS
DNC C1999-109734

consists of two phases, each containing active ingredients, with the first phase releasing ingredients on administration and the second after a set time.
DC B07 C07
IN **DOMS, R**
PA (PHAR-N) PHARMA NATURA PTY LTD
CYC 1
PI ZA 9806934 A 19990428 (199931)* 13
ADT ZA 9806934 A ZA 1998-6934 19980803
PRAI ZA 1997-6996 19970806
AB ZA 9806934 A UPAB: 19990806
NOVELTY - The composition includes two phases, each containing active ingredients selected from vitamins, minerals or mixtures of them. The first phase is adapted to release its active ingredients on administration and the second phase is adapted to release its active ingredients at a time after, or over a period of e.g. 8 hours after, administration.
DETAILED DESCRIPTION - The two phases may be provided as separate layers or one phase may be a discrete region within the other phase in a tablet.
USE - Vitamin and/or mineral tablet suitable for administration as a single daily dose.
ADVANTAGE - Enables vitamins and minerals to be released over e.g. an 8 hour period, avoiding problem of supplement being short lived and avoiding producing an excess of vitamins and minerals which would be eliminated by the kidney in the urine.
Dwg.0/0

L10 ANSWER 7 OF 7 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 1998-298112 [26] WPIDS
DNN N1998-233149 DNC C1998-093073
TI Screening for modulators of amyloid processing in Alzheimer's disease - by measuring amyloid beta peptide(s) in endoplasmic reticulum of NT2N cells, useful to identify agents for Alzheimer's diagnosis and treatment.
DC B04 D16 S03
IN **DOMS, R W**; LEE, V M -; LEE, V M
PA (UYPE-N) UNIV PENNSYLVANIA; (DOMS-I) DOMS R W; (LEEV-I) LEE V M -
CYC 21
PI WO 9821589 A1 19980522 (199826)* EN 26
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA JP US
AU 9871810 A 19980603 (199842)
US 2003186315 A1 20031002 (200365)
ADT WO 9821589 A1 WO 1997-US20212 19971113; AU 9871810 A AU 1998-71810 19971113; US 2003186315 A1 WO 1997-US20212 19971113, US 1999-297877 19990628
FDT AU 9871810 A Based on WO 9821589
PRAI US 1997-55299P 19970808; US 1996-30967P 19961115; US 1999-297877 19990628
AB WO 9821589 A UPAB: 19980701
Method for identifying agents which increase or decrease processing of amyloid precursor protein (APP) into amyloid beta (A beta) peptides, which occur in neuritic plaques and vascular deposits accumulating in the brains of patients with Alzheimer's disease, comprises contacting NT2N cells with a test agent and measuring levels of A beta peptides formed in the endoplasmic reticulum of the cells.
USE - The method is useful to identify agents increasing processing of APP into A beta peptides, useful in (especially early) diagnosis of Alzheimer's disease.
Agents decreasing processing can also be identified, and subsequently administered to inhibit processing in the brains of patients with Alzheimer's disease (all claimed).
Patients suffering from Alzheimer's disease demonstrate neuritic plaques and vascular deposits consisting of amyloid fibrils made up of A beta peptides; A beta is derived from proteolytic processing of APP by several known pathways, the importance of each pathway being thought to be dependant on cell type. Neuronal cells process APP by the beta -secretase pathway, in which beta -secretase cleaves APP at the amino terminus of the A beta domain, releasing a distinct N-terminal fragment (APP beta), then gamma -secretase cleaves APP at alternative sites of the carboxy terminus, generating species of A beta that are either 40 (A beta 40) or 42 amino acids long (A beta 42).
Novel beta -secretase pathways have been identified that produce A beta 42 and APP beta intracellularly in NT2N neurones prior to secretion, and these activities identified to occur in the endoplasmic reticulum (ER)/intermediate compartment of the cell. Discovery of these enzymatic pathways and their location within the ER can be used to design new therapeutic approaches as well as agents which reduce production of A beta peptides.
Alternatively, it may be possible to identify genetic mutations as well as agents which increase this process, for diagnostic purposes. Mutations in both the APP gene and recently identified presenilin genes are believed to cause AD by altering APP processing in ways that lead to

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     Presenilin proteins have been localised to the ER, and the localisation of
     amyloidogenic processing to within the ER of neurones allows the possible
     effects of presenilin genes on APP processing to be examined.
     Dwg.0/0


=> e hoxie j a/in
E1             2      HOXIE B M/IN
E2             1      HOXIE C M/IN
E3             6 --> HOXIE J A/IN
E4             1      HOXIE P/IN
E5             1      HOXIE S W/IN
E6             1      HOXIE V C/IN
E7             1      HOXIMEIER R J/IN
E8            63      HOXMEIER R J/IN
E9             1      HOXTERMAN R/IN
E10           15      HOXTERMANN R/IN
E11            1      HOXTERMANN R H/IN
E12            4      HOXWORTH E/IN

=> s e3
L11            6 "HOXIE J A"/IN

=> s l11 not l10
L12            4 L11 NOT L10

=> d l12,ti,1-4

L12  ANSWER 1 OF 4  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   New nucleic acid encoding a mammalian immunodeficiency virus glycoprotein
     (gp) 120 polypeptide, useful in preparing a composition for treating
     infection caused by simian immunodeficiency virus (SIV), HIV-1 or HIV-2.

L12  ANSWER 2 OF 4  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Anti-immunodeficiency virus antibody - binds to a cellular protein
     required for virus infection, used for treating or preventing infection by
     e.g. HIV or SIV.

L12  ANSWER 3 OF 4  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Detection of thrombin-induced cell activation from presence of receptor
     fragment - used to detect pre-thrombotic states.

L12  ANSWER 4 OF 4  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Detection of thrombin-induced cell activation - by a system to measure a
     cleaved peptide fragment of a thrombin receptor..


=> file medline
COST IN U.S. DOLLARS                              SINCE FILE       TOTAL
                                                       ENTRY     SESSION
FULL ESTIMATED COST                                    40.09       82.31

FILE 'MEDLINE' ENTERED AT 13:12:03 ON 15 OCT 2006

 FILE LAST UPDATED: 14 Oct 2006 (20061014/UP).  FILE COVERS 1950 TO DATE.

 On December 11, 2005, the 2006 MeSH terms were loaded.

 The MEDLINE reload for 2006 is now (26 Feb.) available.  For details
 on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>).
 See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

 OLDMEDLINE is covered back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2006 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> e doms r w/au
E1             2      DOMS P F/AU
E2             3      DOMS R/AU
E3           129 --> DOMS R W/AU
E4            62      DOMS ROBERT W/AU
E5             1      DOMS S R/AU
E6             1      DOMS W/AU
```

```
E8            1     DOMSA IACOB/AU
E9            1     DOMSA L B/AU
E10           1     DOMSA M/AU
E11           1     DOMSA Z/AU
E12           2     DOMSCH C/AU

=> s e2-e4
              3 "DOMS R"/AU
            129 "DOMS R W"/AU
             62 "DOMS ROBERT W"/AU
L13         194 ("DOMS R"/AU OR "DOMS R W"/AU OR "DOMS ROBERT W"/AU)

=> s l13 and (virus-like particle? or VLP? or retroviral vector particle? or RVVP? or human immunodeficiency virus or H
         420269 VIRUS
         385638 LIKE
         125929 PARTICLE?
           2766 VIRUS-LIKE PARTICLE?
                  (VIRUS(W)LIKE(W)PARTICLE?)
           1531 VLP?
          14948 RETROVIRAL
          65832 VECTOR
         125929 PARTICLE?
             35 RETROVIRAL VECTOR PARTICLE?
                  (RETROVIRAL(W)VECTOR(W)PARTICLE?)
              2 RVVP?
        1423747 HUMAN
         124955 IMMUNODEFICIENCY
         420269 VIRUS
          49484 HUMAN IMMUNODEFICIENCY VIRUS
                  (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
         163750 HIV
         123411 MURINE
         188971 LEUKEMIA
         420269 VIRUS
           9157 MURINE LEUKEMIA VIRUS
                  (MURINE(W)LEUKEMIA(W)VIRUS)
           1157 MLV
L14         129 L13 AND (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PARTI
                CLE? OR RVVP? OR HUMAN IMMUNODEFICIENCY VIRUS OR HIV OR MURINE
               LEUKEMIA VIRUS OR MLV)

=> s l14 and (seven-spanning membrane or multiple membrane or multiple transmembrane)
         276904 SEVEN
          17217 SPANNING
         666997 MEMBRANE
              2 SEVEN-SPANNING MEMBRANE
                  (SEVEN(W)SPANNING(W)MEMBRANE)
         490465 MULTIPLE
         666997 MEMBRANE
            239 MULTIPLE MEMBRANE
                  (MULTIPLE(W)MEMBRANE)
         490465 MULTIPLE
          49181 TRANSMEMBRANE
            110 MULTIPLE TRANSMEMBRANE
                  (MULTIPLE(W)TRANSMEMBRANE)
L15           1 L14 AND (SEVEN-SPANNING MEMBRANE OR MULTIPLE MEMBRANE OR MULTIPL
                E TRANSMEMBRANE)

=> d l15,cbib,ab

L15  ANSWER 1 OF 1     MEDLINE on STN
2001022628.    PubMed ID: 11005830.   A biosensor assay for studying
     ligand-membrane receptor interactions: binding of antibodies and HIV-1
     Env to chemokine receptors. Hoffman T L; Canziani G; Jia L; Rucker J;
     Doms R W. (Departments of Pathology and Lab Medicine, Medicine, and
     Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA
     19104, USA. ) Proceedings of the National Academy of Sciences of the
     United States of America, (2000 Oct 10) Vol. 97, No. 21, pp. 11215-20.
     Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States.
     Language: English.
AB   The HIV envelope (Env) protein mediates entry into cells by binding CD4
     and an appropriate coreceptor, which triggers structural changes in Env
     that lead to fusion between the viral and cellular membranes.  The major
     HIV-1 coreceptors are the seven transmembrane domain chemokine receptors
     CCR5 and CXCR4.  The type of coreceptor used by a virus strain is an
     important determinant of viral tropism and pathogenesis, and
     virus-receptor interactions can be therapeutic targets.  However, Envs
     from many virus strains interact with CXCR4 and CCR5 with low affinity
     such that direct study of this important interaction is difficult if not
     impossible using standard cell-surface binding techniques.  We have
     developed an approach that makes it possible to study ligand binding to
     membrane proteins, including Env-coreceptor interactions, using an optical
```

biosensor. CCR5, CXCR4, and other membrane proteins were incorporated into retrovirus particles, which were purified and attached to the biosensor surface. Binding of conformationally sensitive antibodies as well as Env to these receptors was readily detected. The equilibrium dissociation constant for the interaction between an Env derived from the prototype **HIV-1** strain IIIB for CXCR4 was approximately 500 nM, explaining the difficulty in measuring this interaction using standard equilibrium binding techniques. Retroviral pseudotypes represent easily produced, stable, homogenous structures that can be used to present a wide array of single and **multiple membrane**-spanning proteins in a native lipid environment for biosensor studies, thus avoiding the need for detergent solubilization, purification, and reconstitution. The approach should have general applicability and can be used to correlate Env-receptor binding constants to viral tropism and pathogenesis.

```
=> d his

     (FILE 'HOME' ENTERED AT 13:06:04 ON 15 OCT 2006)

     FILE 'USPATFULL' ENTERED AT 13:06:13 ON 15 OCT 2006
                E DOMS ROBERT W/IN
L1           12 S E3
                E HOXIE JAMES A/IN
L2            8 S E3
L3            4 S L2 NOT L1
                E BATES PAUL/IN
L4            6 S E3-E6
L5            5 S L4 NOT (L1 OR L2)
                E HOFFMAN TREVOR L/IN
L6            4 S E3
L7            0 S L6 NOT (L1 OR L2)
                E RUCKER JOSEPH/IN
L8            4 S E3-E5
L9            3 S L8 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 13:10:28 ON 15 OCT 2006
                E DOMS ROBERT W/IN
L10           7 S E1-E2
                E HOXIE J A/IN
L11           6 S E3
L12           4 S L11 NOT L10

     FILE 'MEDLINE' ENTERED AT 13:12:03 ON 15 OCT 2006
                E DOMS R W/AU
L13         194 S E2-E4
L14         129 S L13 AND (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PA
L15           1 S L14 AND (SEVEN-SPANNING MEMBRANE OR MULTIPLE MEMBRANE OR MULT

=> s l14 and (CCR5 or CXCR4 or MCAT? or CXCR2 or CXCR3 or opioid or opsin?)
          3836 CCR5
          3147 CXCR4
           367 MCAT?
           532 CXCR2
           767 CXCR3
         49056 OPIOID
          2249 OPSIN?
L16          75 L14 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID OR
                OPSIN?)

=> s l16 and (opioid or ospin? or bacteriorhodopsin or rhodopsin or porin? or adrenergic receptor)
         49056 OPIOID
             3 OSPIN?
          2390 BACTERIORHODOPSIN
          6435 RHODOPSIN
          3598 PORIN?
        111660 ADRENERGIC
        547362 RECEPTOR
         12791 ADRENERGIC RECEPTOR
                 (ADRENERGIC(W)RECEPTOR)
L17           0 L16 AND (OPIOID OR OSPIN? OR BACTERIORHODOPSIN OR RHODOPSIN OR
                PORIN? OR ADRENERGIC RECEPTOR)

=> s l16 and (virus-like particle or VLP or viral pseudotype?)
        420269 VIRUS
        385638 LIKE
         60599 PARTICLE
           314 VIRUS-LIKE PARTICLE
                 (VIRUS(W)LIKE(W)PARTICLE)
           756 VLP
        353204 VIRAL
          1374 PSEUDOTYPE?
            14 VIRAL PSEUDOTYPE?
```

L18 0 L16 AND (VIRUS-LIKE PARTICLE OR VLP OR VIRAL PSEUDOTYPE?)

```
=> s l16 and py<1998
     11522178 PY<1998
                (PY<19980000)
L19         15 L16 AND PY<1998

=> d l19,cbib,ab,1-15
```

L19 ANSWER 1 OF 15 MEDLINE on STN
1998070822. PubMed ID: 9405683. CD4-independent, **CCR5**-dependent infection of brain capillary endothelial cells by a neurovirulent simian immunodeficiency virus strain. Edinger A L; Mankowski J L; Doranz B J; Margulies B J; Lee B; Rucker J; Sharron M; Hoffman T L; Berson J F; Zink M C; Hirsch V M; Clements J E; **Doms R W**. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.) Proceedings of the National Academy of Sciences of the United States of America, **(1997 Dec 23)** Vol. 94, No. 26, pp. 14742-7. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Brain capillary endothelial cells (BCECs) are targets of CD4-independent infection by **HIV**-1 and simian immunodeficiency virus (SIV) strains in vitro and in vivo. Infection of BCECs may provide a portal of entry for the virus into the central nervous system and could disrupt blood-brain barrier function, contributing to the development of AIDS dementia. We found that rhesus macaque BCECs express chemokine receptors involved in **HIV** and SIV entry including **CCR5**, CCR3, **CXCR4**, and STRL33, but not CCR2b, GPR1, or GPR15. Infection of BCECs by the neurovirulent strain SIV/17E-Fr was completely inhibited by aminooxypentane regulation upon activation, normal T cell expression and secretion in the presence or absence of ligands, but not by eotaxin or antibodies to CD4. We found that the envelope (env) proteins from SIV/17E-Fr and several additional SIV strains mediated cell-cell fusion and virus infection with CD4-negative, **CCR5**-positive cells. In contrast, fusion with cells expressing the coreceptors STRL33, GPR1, and GPR15 was CD4-dependent. These results show that **CCR5** can serve as a primary receptor for SIV in BCECs and suggest a possible CD4-independent mechanism for blood-brain barrier disruption and viral entry into the central nervous system.

L19 ANSWER 2 OF 15 MEDLINE on STN
1998037607. PubMed ID: 9371556. Utilization of chemokine receptors, orphan receptors, and herpesvirus-encoded receptors by diverse human and simian immunodeficiency viruses. Rucker J; Edinger A L; Sharron M; Samson M; Lee B; Berson J F; Yi Y; Margulies B; Collman R G; Doranz B J; Parmentier M; **Doms R W**. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia 19104, USA.) Journal of virology, **(1997 Dec)** Vol. 71, No. 12, pp. 8999-9007. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1) requires both CD4 and a coreceptor to infect cells. Macrophage-tropic (M-tropic) **HIV**-1 strains utilize the chemokine receptor **CCR5** in conjunction with CD4 to infect cells, while T-cell-tropic (T-tropic) strains generally utilize **CXCR4** as a coreceptor. Some viruses can use both **CCR5** and **CXCR4** for virus entry (i.e., are dual-tropic), while other chemokine receptors can be used by a subset of virus strains. Due to the genetic diversity of **HIV**-1, **HIV**-2, and simian immunodeficiency virus (SIV) and the potential for chemokine receptors other than **CCR5** or **CXCR4** to influence viral pathogenesis, we tested a panel of 28 **HIV**-1, **HIV**-2, and SIV envelope (Env) proteins for the ability to utilize chemokine receptors, orphan receptors, and herpesvirus-encoded chemokine receptor homologs by membrane fusion and virus infection assays. While all Env proteins used either **CCR5** or **CXCR4** or both, several also used CCR3. Use of CCR3 was strongly dependent on its surface expression levels, with a larger number of viral Env proteins being able to utilize this coreceptor at the higher levels of surface expression. ChemR1, an orphan receptor recently shown to bind the CC chemokine I309 (and therefore renamed CCR8), was expressed in monocyte and lymphocyte cell populations and functioned as a coreceptor for diverse **HIV**-1, **HIV**-2, and SIV Env proteins. Use of ChemR1/CCR8 by SIV strains was dependent in part on V3 loop sequences. The orphan receptor V28 supported Env-mediated cell-cell fusion by four T- or dual-tropic **HIV**-1 and **HIV**-2 strains. Three additional orphan receptors failed to function for any of the 28 Env proteins tested. Likewise, five of six seven-transmembrane-domain receptors encoded by herpesviruses did not support Env-mediated membrane fusion. However, the chemokine receptor US28, encoded by cytomegalovirus, did support inefficient infection by two **HIV**-1 strains. These findings indicate that additional chemokine receptors can function as **HIV** and SIV coreceptors and that surface expression levels can strongly influence coreceptor use.

L19 ANSWER 3 OF 15 MEDLINE on STN
97477424. PubMed ID: 9334380. A small-molecule inhibitor directed against

the chemokine receptor **CXCR4** prevents its use as an **HIV**-1 coreceptor.
Doranz B J; Grovit-Ferbas K; Sharron M P; Mao S H; Goetz M B; Daar E S; **Doms R W**; O'Brien W A. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia 19104, USA.) The Journal of experimental medicine, **(1997 Oct 20)** Vol. 186, No. 8, pp. 1395-400. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB The chemokine receptor **CXCR4** is the major coreceptor used for cellular entry by T cell- tropic **human immunodeficiency virus** (**HIV**)-1 strains, whereas **CCR5** is used by macrophage (M)-tropic strains. Here we show that a small-molecule inhibitor, ALX40-4C, inhibits **HIV**-1 envelope (Env)-mediated membrane fusion and viral entry directly at the level of coreceptor use. ALX40-4C inhibited **HIV**-1 use of the coreceptor **CXCR4** by T- and dual-tropic **HIV**-1 strains, whereas use of **CCR5** by M- and dual-tropic strains was not inhibited. Dual-tropic viruses capable of using both **CXCR4** and **CCR5** were inhibited by ALX40-4C only when cells expressed **CXCR4** alone. ALX40-4C blocked stromal-derived factor (SDF)-1alpha-mediated activation of **CXCR4** and binding of the monoclonal antibody 12G5 to cells expressing **CXCR4**. Overlap of the ALX40-4C binding site with that of 12G5 and SDF implicates direct blocking of Env interactions, rather than downregulation of receptor, as the mechanism of inhibition. Thus, ALX40-4C represents a small-molecule inhibitor of **HIV**-1 infection that acts directly against a chemokine receptor at the level of Env-mediated membrane fusion.

L19 ANSWER 4 OF 15 MEDLINE on STN
97472350. PubMed ID: 9333175. **CCR5** genotypes in sexually active couples discordant for **human immunodeficiency virus** type 1 infection status. Hoffman T L; MacGregor R R; Burger H; Mick R; **Doms R W**; Collman R G. (Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia 19104-6060, USA.) The Journal of infectious diseases, **(1997 Oct)** Vol. 176, No. 4, pp. 1093-6. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB Persons who are homozygous for the delta32 polymorphism of the **CCR5** chemokine receptor gene are highly protected against **human immunodeficiency virus** type 1 (**HIV**-1) infection. Previous studies described 54 **HIV**-1-discordant couples in whom no virus transmission occurred despite extensive sexual contact. The possible role of the delta32 polymorphism in the lack of **HIV**-1 transmission between these partners was studied. No participants were homozygous for the delta32 allele, but the proportion that was heterozygous was higher among **HIV**-1-seronegative than **HIV**-1-seropositive partners (28% vs. 11%, P = .05). This association was seen in heterosexual couples (P = .03) but not in homosexual couples (P = .74). Among white persons, who are most likely to carry the delta32 allele, 38.9% of **HIV**-1-uninfected and 5.6% of **HIV**-1-infected heterosexual partners were heterozygous (P = .04). These data are consistent with a possible association between the heterozygous delta32 genotype in heterosexual sex partners and partial protection against **HIV**-1 infection, and they emphasize the importance of analyzing different risk groups in studies of host factors that influence infection.

L19 ANSWER 5 OF 15 MEDLINE on STN
97428565. PubMed ID: 9281497. Unwelcomed guests with master keys: how **HIV** uses chemokine receptors for cellular entry. **Doms R W**; Peiper S C. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, 19104, USA.. doms@mail.med.upenn.edu) . Virology, **(1997 Sep 1)** Vol. 235, No. 2, pp. 179-90. Ref: 108. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

L19 ANSWER 6 OF 15 MEDLINE on STN
97404635. PubMed ID: 9261347. Two distinct **CCR5** domains can mediate coreceptor usage by **human immunodeficiency virus** type 1. Doranz B J; Lu Z H; Rucker J; Zhang T Y; Sharron M; Cen Y H; Wang Z X; Guo H H; Du J G; Accavitti M A; **Doms R W**; Peiper S C. (Department of Pathology, University of Pennsylvania, Philadelphia 19104, USA.) Journal of virology, **(1997 Sep)** Vol. 71, No. 9, pp. 6305-14. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The chemokine receptor **CCR5** is the major fusion coreceptor for macrophage-tropic strains of **human immunodeficiency virus** type 1 (**HIV**-1). To define the structures of **CCR5** that can support envelope (Env)-mediated membrane fusion, we analyzed the activity of homologs, chimeras, and mutants of human **CCR5** in a sensitive gene reporter cell-cell fusion assay. Simian, but not murine, homologs of **CCR5** were fully active as **HIV**-1 fusion coreceptors. Chimeras between **CCR5** and divergent chemokine receptors demonstrated the existence of two distinct regions of **CCR5** that could be utilized for Env-mediated fusion, the amino-terminal domain and the extracellular loops. Dual-tropic Env proteins were particularly sensitive to alterations in the **CCR5** amino-terminal domain, suggesting that this domain may play a pivotal role in the evolution of coreceptor usage in vivo. We identified individual residues in both functional regions, Asp-11, Lys-197, and Asp-276, that contribute to coreceptor function. Deletion of a highly conserved

cytoplasmic motif rendered **CCR5** incapable of signaling but did not abrogate its ability to function as a coreceptor, implying the independence of fusion and G-protein-mediated chemokine receptor signaling. Finally, we developed a novel monoclonal antibody to **CCR5** to assist in future studies of **CCR5** expression.

L19 ANSWER 7 OF 15 MEDLINE on STN
97322389. PubMed ID: 9177234. Evolution of **HIV**-1 coreceptor usage through interactions with distinct **CCR5** and **CXCR4** domains. Lu Z; Berson J F; Chen Y; Turner J D; Zhang T; Sharron M; Jenks M H; Wang Z; Kim J; Rucker J; Hoxie J A; Peiper S C; **Doms R W**. (James Graham Brown Cancer Center, University of Louisville, Louisville, KY 40202, USA.) Proceedings of the National Academy of Sciences of the United States of America, **(1997 Jun 10)** Vol. 94, No. 12, pp. 6426-31. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The chemokine receptor **CXCR4** functions as a fusion coreceptor for T cell tropic and dual-tropic **HIV**-1 strains. To identify regions of **CXCR4** that are important for coreceptor function, **CXCR4-CXCR2** receptor chimeras were tested for the ability to support **HIV**-1 envelope (env) protein-mediated membrane fusion. Receptor chimeras containing the first and second extracellular loops of **CXCR4** supported fusion by T tropic and dual-tropic **HIV**-1 and **HIV**-2 strains and binding of a monoclonal antibody to **CXCR4**, 12G5, that blocks **CXCR4**-dependent infection by some virus strains. The second extracellular loop of **CXCR4** was sufficient to confer coreceptor function to **CXCR2** for most virus strains tested but did not support binding of 12G5. Truncation of the **CXCR4** cytoplasmic tail or mutation of a conserved DRY motif in the second intracellular loop did not affect coreceptor function, indicating that phosphorylation of the cytoplasmic tail and the DRY motif are not required for coreceptor function. The results implicate the involvement of multiple **CXCR4** domains in **HIV**-1 coreceptor function, especially the second extracellular loop, though the structural requirements for coreceptor function were somewhat variable for different env proteins. Finally, a hybrid receptor in which the amino terminus of **CXCR4** was replaced by that of **CCR5** was active as a coreceptor for M tropic, T tropic, and dual-tropic env proteins. We propose that dual tropism may evolve in **CCR5**-restricted **HIV**-1 strains through acquisition of the ability to utilize the first and second extracellular loops of **CXCR4** while retaining the ability to interact with the **CCR5** amino-terminal domain.

L19 ANSWER 8 OF 15 MEDLINE on STN
97268687. PubMed ID: 9108095. Differential utilization of **CCR5** by macrophage and T cell tropic simian immunodeficiency virus strains. Edinger A L; Amedee A; Miller K; Doranz B J; Endres M; Sharron M; Samson M; Lu Z H; Clements J E; Murphey-Corb M; Peiper S C; Parmentier M; Broder C C; **Doms R W**. (Department of Pathology, University of Pennsylvania, Philadelphia 19104, USA.) Proceedings of the National Academy of Sciences of the United States of America, **(1997 Apr 15)** Vol. 94, No. 8, pp. 4005-10. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Certain chemokine receptors serve as cofactors for **HIV** type 1 envelope (env)-mediated cell-cell fusion and virus infection of CD4-positive cells. Macrophage tropic (M-tropic) **HIV**-1 isolates use **CCR5**, and T cell tropic (T-tropic) strains use **CXCR4**. To investigate the cofactors used by simian immunodeficiency viruses (SIV), we tested four T-tropic and two M-tropic SIV env proteins for their ability to mediate cell-cell fusion with cells expressing CD4 and either human or nonhuman primate chemokine receptors. Unlike **HIV**-1, both M- and T-tropic SIV envs used **CCR5** but not **CXCR4** or the other chemokine receptors tested. However, by testing a panel of **CCR5**/CCR2b chimeras, we found that the structural requirements for **CCR5** utilization by M-tropic and T-tropic SIV strains were different. T-tropic SIV strains required the second extracellular loop of **CCR5** whereas a closely related M-tropic SIV strain could, like M-tropic **HIV**-1 strains, use the amino-terminal domain of **CCR5**. As few as two amino acid changes in the SIV env V3 domain affected the regions of **CCR5** that were critical for fusogenic activity. Receptor signaling was not required for either fusion or infection. Our results suggest that viral tropism may be influenced not only by the coreceptors used by a given virus strain but also by how a given coreceptor is used.

L19 ANSWER 9 OF 15 MEDLINE on STN
97213996. PubMed ID: 9060685. Role of **CCR5** in infection of primary macrophages and lymphocytes by macrophage-tropic strains of **human immunodeficiency virus**: resistance to patient-derived and prototype isolates resulting from the delta **ccr5** mutation. Rana S; Besson G; Cook D G; Rucker J; Smyth R J; Yi Y; Turner J D; Guo H H; Du J G; Peiper S C; Lavi E; Samson M; Libert F; Liesnard C; Vassart G; **Doms R W**; Parmentier M; Collman R G. (Division of Pulmonary and Critical Care, University of Pennsylvania School of Medicine, Philadelphia 19104, USA.) Journal of virology, **(1997 Apr)** Vol. 71, No. 4, pp. 3219-27. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The alpha-chemokine receptor fusin (CXCR-4) and beta-chemokine receptor **CCR5** serve as entry cofactors for T-cell (T)-tropic and macrophage

(...) tropic [illegible] type 1 [illegible], respectively, when expressed with CD4 in otherwise nonpermissive cells. Some M-tropic and dual-tropic strains can also utilize other beta-chemokine receptors, such as CCR2b and CCR3. A mutation of **CCR5** (delta **ccr5**) was recently found to be common in certain populations and appears to confer protection against **HIV**-1 in vivo. Here, we show that this mutation results in a protein that is expressed intracellularly but not on the cell surface. Primary CD4 T cells from delta **ccr5** homozygous individuals were highly resistant to infection with prototype M-tropic **HIV**-1 strains, including an isolate (YU-2) that uses **CCR5** and CCR3, but were permissive for both a T-tropic strain (3B) and a dual-tropic variant (89.6) that uses CXCR-4, **CCR5**, CCR3, or CCR2b. These cells were also resistant to M-tropic patient isolates but were readily infected by T-tropic patient isolates. Primary macrophages from delta **ccr5** homozygous individuals were also resistant to infection with M-tropic strains, including YU-2, but the dual-tropic strain 89.6 was able to replicate in them even though macrophages are highly resistant to CXCR-4-dependent T-tropic isolates. These data show that **CCR5** is the essential cofactor for infection of both primary macrophages and T lymphocytes by most M-tropic strains of **HIV**-1. They also suggest that CCR3 does not function for **HIV**-1 entry in primary lymphocytes or macrophages, but that a molecule(s) other than **CCR5** can support entry into macrophages by certain virus isolates. These studies further define the cellular basis for the resistance to **HIV**-1 infection of individuals lacking functional **CCR5**.

L19 ANSWER 10 OF 15 MEDLINE on STN
97200271. PubMed ID: 9048206. Chemokine receptors as fusion cofactors for **human immunodeficiency virus** type 1 (**HIV**-1). Doranz B J; Berson J F; Rucker J; **Doms R W**. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia 19104, USA.) Immunologic research, **(1997 Feb)** Vol. 16, No. 1, pp. 15-28. Ref: 79. Journal code: 8611087. ISSN: 0257-277X. Pub. country: United States. Language: English.

AB CD4 is the primary cellular receptor for **human immunodeficiency virus** type 1 (**HIV**-1), but is not sufficient for entry of **HIV**-1 into cells. After a decade-long search, the cellular coreceptors that **HIV**-1 requires in conjunction with CD4 have been identified as members of the chemokine receptor family of seven-transmembrane G-protein coupled receptors. The discovery of distinct chemokine receptors that support entry of T-cell tropic (CXCR-4) and macrophage tropic **HIV**-1 strains (CCR-5) explains the differences in cell tropism between viral strains, the inability of **HIV**-1 to infect most nonprimate cells, and the resistance of a small percentage of the population to **HIV**-1 infection. Further understanding of the role of chemokine receptors in viral entry may also help explain the evolution of more pathogenic forms of the virus, viral transmission, and **HIV**-induced pathogenesis. These recent discoveries will aid the development of strategies for combating **HIV**-1 transmission and spread, the understanding of **HIV**-1 fusion mechanisms, and the possible development of small animal models for **HIV**-1 drug and vaccine testing.

L19 ANSWER 11 OF 15 MEDLINE on STN
97083584. PubMed ID: 8929542. CD4-independent infection by **HIV**-2 is mediated by fusin/**CXCR4**. Endres M J; Clapham P R; Marsh M; Ahuja M; Turner J D; McKnight A; Thomas J F; Stoebenau-Haggarty B; Choe S; Vance P J; Wells T N; Power C A; Sutterwala S S; **Doms R W**; Landau N R; Hoxie J A. (Hematology-Oncology Division, University of Pennsylvania, Philadelphia 19104, USA.) Cell, **(1996 Nov 15)** Vol. 87, No. 4, pp. 745-56. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB Several members of the chemokine receptor family have been shown to function in association with CD4 to permit **HIV**-1 entry and infection. However, the mechanism by which these molecules serve as CD4-associated cofactors is unclear. In the present report, we show that one member of this family, termed Fusin/ **CXCR4**, is able to function as an alternative receptor for some isolates of **HIV**-2 in the absence of CD4. This conclusion is supported by the finding that (1) CD4-independent infection by these viruses is inhibited by an anti-Fusin monoclonal antibody, (2) Fusin expression renders human and nonhuman CD4-negative cell lines sensitive to **HIV**-2-induced syncytium induction and/or infection, and (3) Fusin is selectively down-regulated from the cell surface following **HIV**-2 infection. The finding that one chemokine receptor can function as a primary viral receptor strongly suggests that the **HIV** envelope glycoprotein contains a binding site for these proteins and that differences in the affinity and/or the availability of this site can extend the host range of these viruses to include a number of CD4-negative cell types.

L19 ANSWER 12 OF 15 MEDLINE on STN
97053783. PubMed ID: 8898197. Regions in beta-chemokine receptors **CCR5** and CCR2b that determine **HIV**-1 cofactor specificity. Rucker J; Samson M; Doranz B J; Libert F; Berson J F; Yi Y; Smyth R J; Collman R G; Broder C C; Vassart G; **Doms R W**; Parmentier M. (Department of Pathology and

) Cell, **(1996 Nov 1)** Vol. 87, No. 3, pp. 437-46. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB Macrophage-tropic (M-tropic) **HIV**-1 strains use the beta-chemokine receptor **CCR5**, but not CCR2b, as a cofactor for membrane fusion and infection, while the dual-tropic strain 89.6 uses both. **CCR5**/2b chimeras and mutants were used to map regions of **CCR5** important for cofactor function and specificity. M-tropic strains required either the amino-terminal domain or the first extracellular loop of **CCR5**. A CCR2b chimera containing the first 20 N-terminal residues of **CCR5** supported M-tropic envelope protein fusion. Amino-terminal truncations of **CCR5**/CCR2b chimeras indicated that residues 2-5 are important for M-tropic viruses, while 89.6 is dependent on residues 6-9. The identification of multiple functionally important regions in **CCR5**, coupled with differences in how **CCR5** is used by M- and dual-tropic viruses, suggests that interactions between **HIV**-1 and entry cofactors are conformationally complex.

L19 ANSWER 13 OF 15 MEDLINE on STN

96345670. PubMed ID: 8751444. Resistance to **HIV**-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Samson M; Libert F; Doranz B J; Rucker J; Liesnard C; Farber C M; Saragosti S; Lapoumeroulie C; Cognaux J; Forceille C; Muyldermans G; Verhofstede C; Burtonboy G; Georges M; Imai T; Rana S; Yi Y; Smyth R J; Collman R G; **Doms R W**; Vassart G; Parmentier M. (IRIBHN and Services de Genetique Medicale, Virologie and Immunodeficiences, Universite Libre de Bruxelles, Belgium.) Nature, **(1996 Aug 22)** Vol. 382, No. 6593, pp. 722-5. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **HIV**-1 and related viruses require co-receptors, in addition to CD4, to infect target cells. The chemokine receptor CCR-5 (ref.1) was recently demonstrated to be a co-receptor for macrophage-tropic (M-tropic) **HIV**-1 strains, and the orphan receptor LESTR (also called fusin) allows infection by strains adapted for growth in transformed T-cell lines (T-tropic strains). Here we show that a mutant allele of CCR-5 is present at a high frequency in caucasian populations (allele frequency, 0.092), but is absent in black populations from Western and Central Africa and Japanese populations. A 32-base-pair deletion within the coding region results in a frame shift, and generates a non-functional receptor that does not support membrane fusion or infection by macrophage- and dual-tropic **HIV**-1 strains. In a cohort of **HIV**-1 infected caucasian subjects, no individual homozygous for the mutation was found, and the frequency of heterozygotes was 35% lower than in the general population. White blood cells from an individual homozygous for the null allele were found to be highly resistant to infection by M-tropic **HIV**-1 viruses, confirming that CCR-5 is the major co-receptor for primary **HIV**-1 strains. The lower frequency of heterozygotes in seropositive patients may indicate partial resistance.

L19 ANSWER 14 OF 15 MEDLINE on STN

96323150. PubMed ID: 8709256. A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic **human immunodeficiency virus** type 1 strains. Berson J F; Long D; Doranz B J; Rucker J; Jirik F R; **Doms R W**. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia 19104, USA.) Journal of virology, **(1996 Sep)** Vol. 70, No. 9, pp. 6288-95. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Entry of **human immunodeficiency virus** type 1 (**HIV**-1) into cells requires binding to CD4 and fusion with a cellular membrane. Fusion does not occur in most nonhuman cells even when they express human CD4, indicating that one or more human accessory factors are required for virus infection. Recently, a seven-transmembrane domain protein has been shown to serve as an accessory factor for T-cell-tropic (T-tropic) **HIV**-1 isolates (Y. Feng, C. C. Broder, P. E. Kennedy, and E. A. Berger, Science 272:872-877, 1996). Here we show that expression of this glycoprotein, termed fusin, in murine, feline, simian, and quail cell lines, in conjunction with human CD4, rendered these cells fully permissive for **HIV**-1 envelope glycoprotein (Env)-mediated membrane fusion. Expression of CD4 or fusin alone did not permit fusion. In addition, introduction of fusin and CD4 into a human cell line, U87MG, that is resistant to **HIV**-1 induced syncytium formation and to infection by **HIV**-1 when expressing CD4 alone made this cell line permissive for Env-mediated cell-cell fusion. Fusion was observed only with T-tropic Env proteins. Macrophage-tropic (M-tropic) Env proteins from the SF162, ADA, and Ba-L **HIV**-1 strains did not fuse with cells expressing fusin and CD4, suggesting that M-tropic viruses utilize an accessory molecule other than fusin. Finally, coexpression of fusin and CD4 made both a murine and feline cell line susceptible to virus infection by T-tropic, but not M-tropic, **HIV**-1 strains.

L19 ANSWER 15 OF 15 MEDLINE on STN

96270516. PubMed ID: 8674120. A dual-tropic primary **HIV**-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as

S C; Parmentier M; Collman R G; **Doms R W**. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia 19104, USA.) Cell, **(1996 Jun 28)** Vol. 85, No. 7, pp. 1149-58. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB Here, we show that the beta-chemokine receptor CKR-5 serves as a cofactor for M-tropic **HIV** viruses. Expression of CKR-5 with CD4 enables nonpermissive cells to form syncytia with cells expressing M-tropic, but not T-tropic, **HIV**-1 env proteins. Expression of CKR-5 and CD4 enables entry of a M-tropic, but not a T-tropic, virus strain. A dual-tropic primary **HIV**-1 isolate (89.6) utilizes both Fusin and CKR-5 as entry cofactors. Cells expressing the 89.6 env protein form syncytia with QT6 cells expressing CD4 and either Fusin or CKR-5. The beta-chemokine receptors CKR-3 and CKR-2b support **HIV**-1 89.6 env-mediated syncytia formation but do not support fusion by any of the T-tropic or M-tropic strains tested. Our results suggest that the T-tropic viruses characteristic of disease progression may evolve from purely M-tropic viruses prevalent early in virus infection through changes in the env protein that enable the virus to use multiple entry cofactors.

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L4            6 S E3-E6
L5            5 S L4 NOT (L1 OR L2)
                E HOFFMAN TREVOR L/IN
L6            4 S E3
L7            0 S L6 NOT (L1 OR L2)
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L15           1 S L14 AND (SEVEN-SPANNING MEMBRANE OR MULTIPLE MEMBRANE OR MULT
L16          75 S L14 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID O
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L19          15 S L16 AND PY<1998

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L6            4 S E3
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L14          129 S L13 AND (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PA
L15            1 S L14 AND (SEVEN-SPANNING MEMBRANE OR MULTIPLE MEMBRANE OR MULT
L16           75 S L14 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID O
L17            0 S L16 AND (OPIOID OR OSPIN? OR BACTERIORHODOPSIN OR RHODOPSIN O
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L19           15 S L16 AND PY<1998

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FULL ESTIMATED COST                                     19.85    102.16

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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 12 Oct 2006 (20061012/PD)
FILE LAST UPDATED: 12 Oct 2006 (20061012/ED)
HIGHEST GRANTED PATENT NUMBER: US7120935
HIGHEST APPLICATION PUBLICATION NUMBER: US2006230483
CA INDEXING IS CURRENT THROUGH 10 Oct 2006 (20061010/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 12 Oct 2006 (20061012/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Jun 2006
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Jun 2006


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        177693 PARTICLE?/CLM
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L22  ANSWER 1 OF 17  USPATFULL on STN
```

[illegible] Packaging cells comprising codon optimized gagpol sequences and
lacking lentiviral accessory proteins.
Lee, Jeng-Shin, Lincoln, MA, UNITED STATES
Gray, John T., Memphis, TN, UNITED STATES
Mulligan, Richard C., Cambridge, MA, UNITED STATES
US 2006084093 A1 20060420
APPLICATION: US 2005-204755 A1 20050816 (11)
PRIORITY: US 1998-100063P 19980912 (60)
US 1998-100022P 19980911 (60)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:

1. A packaging cell line for producing viral accessory protein independent HIV-derived **retroviral vector particles**, said method comprising: a) a mammalian cell; b) a first retroviral nucleic acid in the cell which comprises a codon optimized coding sequence for an HIV gagpol; c) a second retroviral nucleic acid in the cell which comprises the coding sequence for a heterologous envelope protein; and d) a third retroviral nucleic acid in the cell which encodes a heterologous therapeutic protein and which comprises HIV cis-acting sequences required for packaging, reverse transcription and integration; wherein said packaging cell line produces HIV-derived **retroviral vector particles**.

2. A packaging cell line comprising: a) a mammalian cell; b) a first retroviral nucleic acid in the cell which comprises a codon optimized coding sequence for an HIV gagpol; and c) a second retroviral nucleic acid in the cell which comprises a nucleotide sequence of interest encoding a heterologous therapeutic protein, said nucleic acid further comprising HIV cis-acting sequences required for packaging, reverse transcription and integration; wherein said packaging cell line produces HIV-derived **retroviral vector particles**.

3. A method of producing a packaging cell line which produces HIV-derived **retroviral vector particles**, comprising co-transfecting mammalian host cells with: a) a first plasmid comprising a codon-optimized nucleotide sequence which encodes HIV gagpol proteins; b) a second plasmid comprising a nucleotide sequence which encodes a heterologous envelope protein; and c) a third plasmid comprising a nucleotide sequence encoding a heterologous therapeutic protein and further comprising HIV cis-acting nucleotide sequences required for packaging, reverse transcription and integration; thereby producing a packaging cell line which produces HIV-derived **retroviral vector particles**.

4. A method of producing HIV-derived **retroviral vector particles**, said method comprising: a) co-transfecting mammalian host cells with: i) a first plasmid comprising a codon-optimized nucleotide sequence which encodes HIV gagpol proteins; ii) a second plasmid comprising a nucleotide sequence which encodes a heterologous envelope protein; and iii) a third plasmid comprising a nucleotide sequence encoding a heterologous therapeutic protein and further comprising HIV cis-acting sequences required for packaging, reverse transcription and integration; thereby producing transfected cells; b) maintaining the transfected cells under conditions suitable for production of virus particles; and c) recovering the virus particles produced in step b).

5. A packaging cell line comprising: a) a mammalian cell; b) a first retroviral nucleic acid in the cell, said nucleic acid comprising a codon optimized coding sequence for a lentivirus gagpol; c) a second retroviral nucleic acid in the cell which comprises the coding sequence for a heterologous envelope protein; and d) a third retroviral nucleic acid in the cell, said nucleic acid encoding a heterologous therapeutic protein and said nucleic acid comprising lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

6. A packaging cell line comprising: a) a mammalian cell; b) a first retroviral nucleic acid in the cell, said nucleic acid comprising a codon optimized coding sequence for lentivirus gagpol; and c) a second retroviral nucleic acid in the cell, said nucleic acid encoding a heterologous therapeutic protein, and said nucleic acid comprising lentivirus cis-acting nucleotide sequences required for packaging, reverse transcription and integration.

7. A method of producing a packaging cell line for producing lentivirus-derived **retroviral vector particles**, said method comprising co-transfecting mammalian host cells with: a) a first plasmid comprising a codon optimized nucleotide sequence which encodes lentivirus gagpol proteins; b) a second plasmid comprising a nucleotide sequence which encodes a heterologous envelope protein; and c) a third plasmid comprising a nucleotide sequence encoding a heterologous therapeutic protein and further comprising lentivirus cis-acting nucleic acid sequences required for packaging, reverse transcription and

integration; thereby producing a packaging cell line which produces lentivirus-derived **retroviral vector particles**.

8. A method of producing lentivirus-derived **retroviral vector particles,** said method comprising: a) co-transfecting mammalian host cells with: i) a first plasmid comprising a codon-optimized nucleotide sequence which encodes lentivirus gagpol proteins; ii) a second plasmid comprising a nucleotide sequence which encodes a heterologous envelope protein; and iii) a third plasmid comprising a nucleotide sequence encoding a heterologous therapeutic protein and further comprising lentivirus cis-acting sequences required for packaging, reverse transcription and integration; thereby producing transfected cells; b) maintaining the transfected cells under conditions suitable for production of virus particles; and c) recovering the virus particles produced in step b).

9. HIV-derived **retroviral vector particles** having no viral accessory proteins, said particles produced by a method comprising: a) co-transfecting mammalian host cells with: i) a first plasmid comprising a codon optimized coding sequence for HIV gagpol proteins but not comprising DNA sequences encoding HIV accessory proteins or constitutive transport elements; ii) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and iii) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration; thereby producing transfected cells; and b) maintaining the transfected cells under conditions suitable for production of virus particles.

10. The HIV-derived retroviral particles of claim 9 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

11. The HIV-derived retroviral particles of claim 9 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

12. The HIV-derived retroviral particles of claim 9 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

13. Lentivirus-derived **retroviral vector particles** having no viral accessory proteins, said particles produced by the method comprising: a) co-transfecting mammalian host cells with: i) a first plasmid comprising a codon optimized DNA sequence which encodes lentivirus gagpol proteins but not comprising DNA sequences encoding lentivirus accessory proteins or constitutive transport elements; ii) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and iii) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration; thereby producing transfected cells; and b) maintaining the transfected cells under conditions suitable for production of virus particles.

14. The **retroviral vector particles** of claim 13 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

15. The **retroviral vector particles** of claim 13 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

16. The **retroviral vector particles** of claim 13 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

17. Isolated DNA encoding a co don optimized HIV gagpol.

18. Isolated DNA encoding a codon optimized HIV gag.

19. Isolated DNA of claim 18 comprising SEQ ID NO:4.

20. Isolated DNA encoding a codon optimized HIV pol.

21. Isolated DNA of claim 20 comprising SEQ ID NO:10.

22. A method of introducing a DNA sequence of interest into a mammal, said method comprising introducing into said mammal a viral accessory protein independent HIV-derived **retroviral vector particle** comprising the DNA sequence of interest.

23. The method of claim 22 wherein the mammal is a human.

24. The method of claim 22 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

25. A method of introducing a DNA sequence of interest into a mammal, said method comprising: a) introducing into cells a viral accessory protein independent HIV-derived **retroviral vector particle** comprising the DNA sequence of interest; and b) introducing the cells obtained in step a) into the mammal, thereby introducing the DNA sequence of interest into the mammal.

26. The method of claim 25 wherein the mammal is a human.

27. The method of claim 25 wherein the DNA sequence of interest is a heterologous therapeutic protein.

28. The method of claim 25 wherein the cells of step a) are obtained from the mammal.

L22 ANSWER 2 OF 17 USPATFULL on STN
2006:40224 Immunogenic compositions for Chlamydia trachomatis.
Grandi, Guido, Milano, ITALY
Ratti, Guilio, Siena, ITALY
Bonci, Alessandra, Siena, ITALY
Finco, Oretta, Castelnuovo Berardenga, ITALY
Chiron Corporation, Emeryville, CA, UNITED STATES (U.S. corporation)
US 2006034871 A1 20060216
APPLICATION: US 2004-18868 A1 20041222 (11)
PRIORITY: GB 2003-15020 20030626
GB 2004-2236 20040202
US 2003-497649P 20030825 (60)
US 2004-576375P 20040601 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An immunogenic composition comprising a combination of Chlamydia trachomatis antigens, the combination comprising at least one Chlamydia trachomatis antigen associated with elementary bodies of Chlamydia trachomatis and at least one Chlamydia trachomatis antigen associated with reticulate bodies of Chlamydia trachomatis.

2. The immunogenic composition of claim 1 further comprising a TH1 adjuvant and a TH2 adjuvant.

3. The immunogenic composition of claim 2 wherein said TH1 adjuvant elicits an enhanced cell-mediated immune response.

4. The immunogenic composition of claim 2 wherein said TH2 adjuvant elicits an enhanced antibody response.

5. The immunogenic composition of claim 2 wherein said TH1 adjuvant is selected from the group consisting of saponin formulations, virosomes, **virus like particles,** non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), and immunostimulatory oligonucleotides.

6. The immunogenic composition of claim 2 wherein said TH2 adjuvant is selcted from the group consisting of mineral containing compositions, oil-emulsions, ADP-ribosylating toxins, and detoxified derivatives of ADP-ribosylating toxins.

7. The immunogenic composition of claim 2 wherein said TH1 adjuvant is an immunostimulatory oligonucleotide containing a CpG motif.

8. The immunogenic composition of claim 2 wherein said TH2 adjuvant is an aluminum salt.

9. The immunogenic composition of claim 1 wherein said at least one Chlamydia trachomatis antigen associated with reticulate bodies is a Type III Secretion System (TTSS) effector protein.

10. The immunogenic composition of claim 9 wherein said Type III Secretion System (TTSS) effector protein is an Inclusion Membrane Associated protein.

11. The immunogenic composition of claim 1 wherein at least one Chlamydia trachomatis antigen associated with reticulate bodies is a Type III Secretion System (TTSS) effector protein, said Type III Secretion System (TTSS) effector protein being a non-Inclusion Membrane Associated protein.

12. An immunogenic composition comprising a combination of Chlamydia trachomatis antigens, the combination comprising at least one Chlamydia trachomatis antigen of a first antigen group and at least one Chlamydia trachomatis antigen of a second antigen group, said first antigen group comprising a Type III secretion system (TTSS) protein and said second antigen group comprising a Type III secretion system (TTSS) effector

protein.

13. The immunogenic composition of claim 12 wherein said Type III secretion system (TTSS) protein is LcrE protein.

14. The immunogenic composition of claim 12 wherein said Type III secretion system (TTSS) effector protein is an Inclusion Membrane Associated protein.

15. The immunogenic composition of claim 12 wherein said Type III secretion system (TTSS) effector protein is a non-Inclusion Membrane protein.

16. The immunogenic composition of claim 12 further comprising a TH1 adjuvant and a TH2 adjuvant.

17. The immunogenic composition of claim 12 wherein said TH1 adjuvant is an oligonucleotide comprising a CpG motif and said TH2 adjuvant is an aluminum salt.

18. An immunogenic composition comprising a combination of Chlamydia trachomatis antigens comprising at least one Chlamydia trachomatis antigen that is conserved over at least two serovars.

19. The immunogenic composition of claim 18 wherein the at least two serovars are selected from the group consisting of serovars D, E, F, G, H, I, J, and K.

20. The immunogenic composition of claim 18 wherein said combination of Chlamydia trachomatis antigens comprises at least one Chlamydia trachomatis antigen of a first serovar of Chlamydia trachomatis and at least one Chlamydia trachomatis antigen of a second serovar of Chlamydia trachomatis.

21. The immunogenic composition of claim 20 wherein the first serovar is selected from the group consisting of serovar D, E, F, G, H, I, J, and K.

22. The immunogenic composition of claim 20 wherein the first serovar is selected from the group consisting of serovar D, E, F, G, H, I, J, and K and the second serovar is selected from the group consisting of serovar D, E, F, G, H, I, J, and K, the second serovar being a different serovar from the first serovar.

23. The immunogenic composition of claim 20 wherein the first serovar is selected from the group consisting of serovar D, E, F, G, H, I, J, and K and the second serovar is selected from the group consisting of serovar A, B, Ba, C, L1, L2, and L3.

24. The immunogenic composition of claim 20 wherein the first serovar is selected from the group consisting of serovar A, B, Ba, C, L1, L2, and L3 and the second serovar is selected from the group consisting of serovar A, B, Ba, C, L1, L2, and L3, the second serovar being a different serovar from the first serovar.

25. An immunogenic composition comprising a combination of Chlamydia trachomatis antigens, the combination eliciting a Chlamydia trachomatis specific TH1 immune response and a Chlamydia trachomatis specific TH2 immune response.

26. The immunogenic composition of claim 25 further comprising a TH1 adjuvant and a TH2 adjuvant.

27. The immunogenic composition of claim 26 wherein said TH1 adjuvant elicits an enhanced cell-mediated immune response.

28. The immunogenic composition of claim 26 wherein said TH2 adjuvant elicits an enhanced antibody response.

29. The immunogenic composition of claim 26 wherein said TH1 adjuvant is selected from the group consisting of saponin formulations, virosomes, **virus like particles,** non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), and immunostimulatory oligonucleotides.

30. The immunogenic composition of claim 26 wherein said TH2 adjuvant is selcted from the group consisting of mineral containing compositions, oil-emulsions, ADP-ribosylating toxins, and detoxified derivatives of ADP-ribosylating toxins.

31. The immunogenic composition of claim 26 wherein said TH1 adjuvant is an immunostimulatory oligonucleotide containing a CpG motif.

32. The immunogenic composition of claim 26 wherein said TH2 adjuvant is

an aluminum salt.

33. A method of eliciting a Chlamydia trachomatis specific immune response comprising administering an effective amount of the immunogenic composition of any one of claim 1.

34. The method of claim 33 wherein the immunogenic composition comprises a TH1 adjuvant and a TH2 adjuvant.

35. The method of claim 34 wherein said TH1 adjuvant elicits an enhanced cell-mediated response and the TH2 adjuvant elicits an enhanced antibody response.

36. The method of claim 35 wherein said TH1 adjuvant is selected from the group consisting of saponin formulations, virosomes, **virus like particles**, non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), and immunostimulatory oligonucleotides and said TH2 adjuvant is selected from the group consisting of mineral containing compositions; oil-emulsions, ADP-ribosylating toxins, and detoxified derivatives of ADP-ribosylating toxins.

37. The method of claim 36 wherein said TH1 adjuvant is an immunostimulatory oligonucleotide containing a CpG motif and said TH2 adjuvant is an aluminum salt.

38. A method of monitoring the efficacy of treatment of a patient infected with Chlamydia trachomatis comprising determining the level of Chlamydia trachomatis specific antibody in the patient after administration of the immunogenic composition of claim 1 to the patient.

39. The method of claim 37 wherein a post-immunization level of of Chlamydia trachomatis specific antibody is measured in the serum of the patient.

40. The method of claim 38 wherein the Chlamydia trachomatis specific antibody is IgG1 or IgG2a.

41. The method of claim 36 wherein a post-immunzation level of Chlamydia trachomatis specific antibody is measured in the mucosal secretions of the patient.

42. The method of claim 40 wherein the Chlamydia trachomatis specific antibody is IgA.

43. A kit comprising: an immunogenic composition of claim 1; a TH1 adjuvant; a TH2 adjuvant; and instructions.

44. The kit of claim 42 wherein the immunogenic composition comprises at least one Chlamydia trachomatis antigen of a first antigen group and the at least one Chlamydia trachomatis antigen of a second antigen group, said first antigen group comprising a Type III secretion system (TTSS) rprotein and said second antigen group comprising a Type III secretion system (TTSS) effectorprotein or a fragment thereof.

45. The kit of claim 44 wherein said first antigen group comprises an LcrE protein and said second antigen group comprises an Inclusion Membrane Associated protein.

L22 ANSWER 3 OF 17 USPATFULL on STN
2005:270536 Packaging cells comprising codon-optimized gagpol sequences and lacking lentiviral accessory proteins.
Gray, John T., Memphis, TN, UNITED STATES
Lee, Jeng-Shin, Lincoln, MA, UNITED STATES
Mulligan, Richard C., Cambridge, MA, UNITED STATES
The Children's Medical Center Corp., Boston, MA, UNITED STATES (U.S. corporation)
US 6958226 B1 20051025
APPLICATION: US 1999-393795 19990910 (9)
PRIORITY: US 1998-100063P 19980912 (60)
US 1998-100022P 19980911 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A packaging cell line comprising: a) a mammalian cell; b) a first retroviral nucleotide sequence in the cell which comprises a codon optimized coding sequence for a HIV gagpol and lacks coding sequences for HIV accessory proteins, Rev response element and constitutive transport elements; c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein; and d) a third retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration, wherein

said packaging cell line produces a HIV-derived **retroviral vector particle**.

2. A packaging cell line of claim 1 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

3. A packaging cell line of claim 1 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

4. A packaging cell line comprising: a) a mammalian cell; b) a first retroviral nucleotide sequence in the cell which comprises a codon optimized coding sequence for a HIV gagpol and lacks coding sequences for HIV accessory proteins, Rev response element and constitutive transport elements; and a second retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

5. A packaging cell line comprising: a) a mammalian cell; b) a first retroviral nucleotide sequence in the cell which comprises a codon optimized coding sequence for a HIV gagpol and lacks coding sequences for HIV accessory proteins, Rev response element and constitutive transport elements; and c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein.

6. A method of producing a packaging cell line which produces a HIV-derived **retroviral vector particle**, comprising co-transfecting mammalian host cells with: a) a first plasmid comprising a codon optimized DNA sequence which encodes HIV gagpol proteins and lacks DNA sequences encoding HIV accessory proteins, Rev response element and constitutive transport elements; b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration, thereby producing a packaging cell line which produces a HIV-derived **retroviral vector particle**.

7. A method of claim 6 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

8. A method of claim 6 wherein the heterologous envelope protein is the amphotropic envelope protien of the Moloney leukemia virus.

9. A method of producing a HIV-derived **retroviral vector particle** comprising the steps of: a) co-transfecting mammalian host cells with: i) a first plasmid comprising a codon optimized DNA sequence which encodes HIV gagpol proteins and lacks DNA sequences encoding HIV accessory proteins, Rev response element and constitutive transport elements; ii) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and iii) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration, b) maintaining the transfected cells under conditions suitable for virus particle production; and c) recovering virus particle produced in step b).

10. A method of claim 9 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

11. A method of claim 9 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

12. A packaging cell line comprising: a) a mammalian cell; b) a first retroviral nucleotide sequence in the cell which comprises a codon optimized coding sequence for a lentivirus gagpol and lacks coding sequences for lentivirus accessory proteins, Rev response element and constitutive transport elements; c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein; and d) a third retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration, wherein said packaging cell line produces a lentivirus-derived **retroviral vector particle**.

13. A packaging cell line of claim 12 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

14. A packaging cell line of claim 12 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

15. A packaging cell line comprising: a) a mammalian cell; b) a first retroviral nucleotide sequence in the cell which comprises a codon optimized coding sequence for lentivirus gagpol and lacks coding sequences for lentivirus accessory proteins, Rev response element and

constitutive transport elements; and c) a second retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

16. A packaging cell line comprising: a) a mammalian cell; b) a first retroviral nucleotide sequence in the cell which comprises a codon optimized coding sequence for lentivirus gagpol and lacks coding sequences for lentivirus accessory proteins, Rev response element and constitutive transport elements; and c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein.

17. A method of producing a packaging cell line which produces a lentivirus-derived **retroviral vector particle**, comprising co-transfecting mammalian host cells with: a) a first plasmid comprising a codon optimized DNA sequence which encodes lentivirus gagpol proteins and lacks DNA sequences encoding lentivirus accessory proteins, Rev response element and constitutive transport elements; b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration, thereby producing a packaging cell line which produces a lentivirus-derived **retroviral vector particle**.

18. A method of claim 17 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

19. A method of claim 17 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

20. A method of producing a lentivirus-derived **retroviral vector particle** comprising the steps of: a) co-transfecting mammalian host cells with: i) a first plasmid comprising a codon optimized DNA sequence which encodes lentivirus gagpol proteins and lacks DNA sequences encoding lentivirus accessory proteins, Rev response element and constitutive transport elements; ii) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and iii) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration, b) maintaining the transfected cells under conditions suitable for virus particle production; and c) recovering virus particle produced in step b).

21. A method of claim 20 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

22. A method of claim 20 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

L22 ANSWER 4 OF 17 USPATFULL on STN
2005:254311 Chlamydia antigens and corresponding DNA fragments and uses thereof

Murdin, Andrew D., Richmond Hill, CANADA
Oomen, Raymond P., Aurora, CANADA
Wang, Joe, Toronto, CANADA
Dunn, Pamela, Woodbridge, CANADA
Aventis Pasteur Limited (non-U.S. corporation)
US 2005220805 A1 20051006
APPLICATION: US 2005-142306 A1 20050602 (11)
PRIORITY: US 1999-154652P 19990920 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A vaccine composition comprising a protein and a compound, wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:2, and wherein the compound facilitates delivery and/or enhance an immune response to the part of the protein having the amino acid sequence set forth in SEQ ID No:2.

2. The composition according to claim 1 wherein the protein is a fusion protein comprising the amino acid sequence set forth in SEQ ID NO:2 fused with a heterologous polypeptide.

3. The composition according to claim 1 wherein the heterologous polypeptide is a peptide tail for purifying the protein.

4. The vaccine composition according to claim 1 wherein the compound is a liposome.

5. The composition according to claim 4 wherein the liposome is at least one liposome selected from the group consisting of neutral liposomes,

anionic liposomes, microspheres, ISCOMS, and **virus-like particles (VLPs)**.

6. The composition according to claim 1 wherein the compound is an adjuvant.

7. The composition according to claim 6 which is suitable for parenteral administration.

8. The composition according to claim 7 wherein the adjuvant is at least one adjuvant selected from the group consisting of an aluminum compound, RIBI, polyphosphazene, DC-chol (3b-(N--(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol and QS-21.

9. The composition according to claim 8 wherein the adjuvant is aluminum hydroxide, aluminum phosphate, or aluminum hydroxy phosphate.

10. The composition according to claim 6 which is suitable for mucosal administration.

11. The composition according to claim 10 wherein the adjuvant is at least one adjuvant selected from the group consisting of bacterial toxin, bacterial monophosphoryl lipid A (MPLA), saponin, polylactide glycolide (PLGA) microsphere, polyphosphazene, DC-chol (3b-(N--(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol, and QS-21.

12. The composition according to claim 11 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of cholera toxin (CT), E. Coli heat-labile toxin (LT), Clostridium difficile toxin A, pertussis toxin (PT), and combinations, subunits, toxoids, or mutants thereof that retain adjuvant activity and/or have reduced toxicity.

13. The composition according to claim 12 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of native cholera toxin subunit B (CTB), Arg-7-Lys CT mutant, Arg-192-Gly LT mutant, Arg-9-Lys PT mutant, Glu-129-Gly PT mutant, Ser-63-Lys LT mutant, Ala-69-Gly LT mutant, Glu-110-Asp LT mutant, and Glu-112-Asp LT mutant.

14. The composition according to claim 11 wherein the adjuvant is bacterial monophosphoryl lipid A (MPLA) of E. coli, Salmonella minnesota, Salmonella typhimurium, or Shigella flexneri.

15. The composition according to claim 1 in unit dosage form.

16. A vaccine composition comprising a protein in unit dosage form, wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:2.

17. The composition according to claim 16, further comprising a compound that facilitates delivery and/or enhance an immune response to the part of the protein having the amino acid sequence set forth in SEQ ID NO:2.

18. The composition according to claim 17 wherein the compound is a liposome.

19. The composition according to claim 18 wherein the liposome is at least one liposome selected from the group consisting of neutral liposomes, anionic liposomes, microspheres, ISCOMS, and **virus-like-particles (VLPs)**.

20. The composition according to claim 16, further comprising an adjuvant.

21. The composition according to claim 20 which is suitable for parenteral administration.

22. The composition according to claim 21 wherein the adjuvant is at least one adjuvant selected from the group consisting of an aluminum compound, RIBI, polyphosphazene, DC-chol (3b-(N--(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol and QS-21.

23. The composition according to claim 22 wherein the adjuvant is aluminum hydroxide, aluminum phosphate, or aluminum hydroxy phosphate.

24. The composition according to claim 23 which is suitable for mucosal administration.

25. The composition according to claim 24 wherein the adjuvant is at least one adjuvant selected from the group consisting of bacterial toxin, bacterial monophosphoryl lipid A (MPLA), saponin, polylactide glycolide (PLGA) microsphere, polyphosphazene, DC-chol (3b-(N--(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol, and QS-21.

26. The composition according to claim 25 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of cholera toxin (CT), E. coli heat-labile toxin (LT), Clostridium difficile toxin A, pertussis toxin (PT), and combinations, subunits, toxoids, or mutants thereof that retain adjuvant activity and/or have reduced toxicity.

27. The composition according to claim 26 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of native cholera toxin subunit B (CTB), Arg-7-Lys CT mutant, Arg-192-Gly LT mutant, Arg-9-Lys PT mutant, Glu-129-Gly PT mutant, Ser-63-Lys LT mutant, Ala-69-Gly LT mutant, Glu-110-Asp LT mutant, and Glu-112-Asp LT mutant.

28. The composition according to claim 25 wherein the adjuvant is bacterial monophosphoryl lipid A (MPLA) of E. coli, Salmonella minnesota, Salmonella typhimurium, or Shigella flexneri.

29. The composition according to claim 16, further comprising an additional Chlamydia polypeptide that enhances an immune response to the part of the protein having the amino acid sequence set forth in SEQ ID NO:2.

30. A vaccine composition comprising a protein fused to a heterologous polypeptide having adjuvant activity, wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:2.

31. The vaccine composition according to claim 30 wherein the heterologous polypeptide having adjuvant activity is suitable as an adjuvant for parenteral administration.

32. The vaccine composition according to claim 30 wherein the heterologous polypeptide having adjuvant activity is suitable as an adjuvant for mucosal administration.

33. The vaccine composition according to claim 32 wherein the heterologous polypeptide is subunit B of cholera toxin (CTB) or subunit B of E. coli heat-labile toxin (LTB).

34. The vaccine composition according to claim 30 wherein the heterologous polypeptide is a strong T-cell epitope and/or a strong B-cell epitope.

35. The vaccine composition according to claim 34 wherein the heterologous polypeptide is a strong T-cell epitope and/or a strong B-cell epitope from hepatitis B virus core antigen.

36. The vaccine composition according to claim 30 further comprising a compound that facilitates delivery and/or enhance the immune response to the part of the protein having the amino acid sequence set forth in SEQ ID NO:2.

37. The vaccine composition according to claim 36 wherein the compound that facilitates delivery and/or enhance the immune response is a liposome.

38. The vaccine composition according to claim 30 further comprising an adjuvant.

39. The vaccine composition according to claim 30 in unit dosage form.

40. A method for treating or preventing a Chlamydia infection, the method comprising the step of administering to a subject the vaccine composition as defined in claim 1.

41. A method for treating or preventing a Chlamydia infection, the method comprising the step of administering to a subject the vaccine composition as defined in claim 16.

42. A method for treating or preventing a Chlamydia infection, the method comprising the step of administering to a subject the vaccine composition as defined in claim 30.

43. A method for treating or preventing a Chlamydia infection, the method comprising the step of administering to a subject the vaccine composition as defined in claim 10, and further comprising the step of administering to the subject an antibiotic, an antacid, sucralfate, a cytokine immunomodulator or a combination thereof.

44. The method according to claim 43 wherein the antibiotic is a macrolide, a tetracycline, or a derivative thereof.

45. The method according to claim 43 wherein the antibiotic is

azithromycin or doxicyclin, and wherein the immunomodulator is interleukin-2 (IL-2), interleukin-12 (IL-12), or a steroid.

46. A vaccination kit comprising the vaccine composition as defined in claim 1 and instructions for its use in vaccinating a subject against Chlamydia infection.

47. A vaccination kit comprising the vaccine composition as defined in claim 16 and instructions for its use in vaccinating a subject against Chlamydia infection.

48. A vaccination kit comprising the vaccine composition as defined in claim 30 and instructions for its use in vaccinating a subject against Chlamydia infection.

49. A vaccination kit comprising the vaccine composition as defined in claim 10, at least one compound selected from the group consisting of an antibiotic, an antacid, sucralfate, a cytokine immunomodulator, and instructions for using the composition and compound in vaccinating a subject against Chlamydia infection.

50. The kit according to claim 49 wherein the antibiotic is a macrolide, a tetracycline, or a derivative thereof.

51. The kit according to claim 49 wherein the antibiotic is azithromycin or doxicyclin, and wherein the immunomodulator is interleukin-2 (IL-2), interleukin-12 (IL-12), or a steroid.

L22 ANSWER 5 OF 17 USPATFULL on STN
2005:233114 Chlamydia antigens and vaccine uses of the protein.
Murdin, Andrew D., Richmond Hill, CANADA
Oomen, Raymond P., Aurora, CANADA
Wang, Joe, Toronto, CANADA
Dunn, Pamela, Woodbridge, CANADA
Aventis Pasteur Limited (non-U.S. corporation)
US 2005202048 A1 20050915
APPLICATION: US 2005-118381 A1 20050502 (11)
PRIORITY: US 1999-132270P 19990503 (60)
US 1999-141276P 19990630 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A vaccine composition comprising a protein and a compound, wherein the protein comprises the amino acid sequence represented by the formula: X--Y wherein X represents the N-terminal residue of the amino acid sequence from SEQ ID NO:2 and is any amino acid from position 1 to 69 of SEQ ID NO:2; wherein Y represents the C-terminal residue of the amino acid sequence from SEQ ID NO:2 and is any amino acid from position 452 to 651 of SEQ ID NO:2; and wherein the compound facilitates delivery and/or enhance an immune response to the part of the protein having the amino acid sequence represented by the formula X--Y.

2. The vaccine composition according to claim 1 wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:2.

3. The vaccine composition according to claim 1 wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:4.

4. The vaccine composition according to claim 1 wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:6.

5. The composition according to claim 1 wherein the protein is a fusion protein comprising the amino acid sequence represented by the formula X--Y, fused with a heterologous polypeptide.

6. The composition according to claim 1 wherein the heterologous polypeptide is a peptide tail for purifying the protein.

7. The vaccine composition according to claim 1 wherein the compound is a liposome.

8. The composition according to claim 7 wherein the liposome is at least one liposome selected from the group consisting of neutral liposomes, anionic liposomes, microspheres, ISCOMS, and **virus-like-particles (VLPs)**.

9. The composition according to claim 1 wherein the compound is an adjuvant.

10. The composition according to claim 9 which is suitable for parenteral administration.

11. The composition according to claim [illegible] wherein the adjuvant is at least one adjuvant selected from the group consisting of an aluminum compound, RIBI, polyphosphazene, DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol and QS-21.

12. The composition according to claim 11 wherein the adjuvant is aluminum hydroxide, aluminum phosphate, or aluminum hydroxy phosphate.

13. The composition according to claim 9 which is suitable for mucosal administration.

14. The composition according to claim 13 wherein the adjuvant is at least one adjuvant selected from the group consisting of bacterial toxin, bacterial monophosphoryl lipid A (MPLA), saponin, polylactide glycolide (PLGA) microsphere, polyphosphazene, DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol, and QS-21.

15. The composition according to claim 14 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of cholera toxin (CT), E. coli heat-labile toxin (LT), Clostridium difficile toxin A, pertussis toxin (PT), and combinations, subunits, toxoids, or mutants thereof that retain adjuvant activity and/or have reduced toxicity.

16. The composition according to claim 15 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of native cholera toxin subunit B (CTB), Arg-7-Lys CT mutant, Arg-192-Gly LT mutant, Arg-9-Lys PT mutant, Glu-129-Gly PT mutant, Ser-63-Lys LT mutant, Ala-69-Gly LT mutant, Glu-110-Asp LT mutant, and Glu-112-Asp LT mutant.

17. The composition according to claim 14 wherein the adjuvant is bacterial monophosphoryl lipid A (MPLA) of E. coli, Salmonella minnesota, Salmonella typhimurium, or Shigella flexneri.

18. The composition according to claim 1 in unit dosage form.

19. A vaccine composition comprising a protein in unit dosage form, wherein the protein comprises the amino acid sequence represented by the formula: X--Y wherein X represents the N-terminal residue of the amino acid sequence from SEQ ID NO:2 and is any amino acid from position 1 to 69 of SEQ ID NO:2; wherein Y represents the C-terminal residue of the amino acid sequence from SEQ ID NO:2 and is any amino acid from position 452 to 651 of SEQ ID NO:2.

20. The vaccine composition according to claim 19 wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:2.

21. The vaccine composition according to claim 19 wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:4.

22. The vaccine composition according to claim 19 wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:6.

23. The composition according to claim 19, further comprising a compound that facilitates delivery and/or enhance an immune response to the part of the protein having the amino acid sequence represented by the formula X--Y.

24. The composition according to claim 23 wherein the compound is a liposome.

25. The composition according to claim 24 wherein the liposome is at least one liposome selected from the group consisting of neutral liposomes, anionic liposomes, microspheres, ISCOMS, and **virus-like-particles (VLPs)**.

26. The composition according to claim 19, further comprising an adjuvant.

27. The composition according to claim 26 which is suitable for parenteral administration.

28. The composition according to claim 27 wherein the adjuvant is at least one adjuvant selected from the group consisting of an aluminum compound, RIBI, polyphosphazene, DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol and QS-21.

29. The composition according to claim 28 wherein the adjuvant is aluminum hydroxide, aluminum phosphate, or aluminum hydroxy phosphate.

30. The composition according to claim 29 which is suitable for mucosal administration.

31. The composition according to claim 30 wherein the adjuvant is at least one adjuvant selected from the group consisting of bacterial toxin, bacterial monophosphoryl lipid A (MPLA), saponin, polylactide glycolide (PLGA) microsphere, polyphosphazene, DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol, and QS-21.

32. The composition according to claim 31 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of cholera toxin (CT), E. coli heat-labile toxin (LT), Clostridium difficile toxin A, pertussis toxin (PT), and combinations, subunits, toxoids, or mutants thereof that retain adjuvant activity and/or have reduced toxicity.

33. The composition according to claim 32 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of native cholera toxin subunit B (CTB), Arg-7-Lys CT mutant, Arg-192-Gly LT mutant, Arg-9-Lys PT mutant, Glu-129-Gly PT mutant, Ser-63-Lys LT mutant, Ala-69-Gly LT mutant, Glu-110-Asp LT mutant, and Glu-112-Asp LT mutant.

34. The composition according to claim 31 wherein the adjuvant is bacterial monophosphoryl lipid A (MPLA) of E. coli, Salmonella minnesota, Salmonella typhimurium, or Shigella flexneri.

35. The composition according to claim 19, further comprising an additional Chlamydia polypeptide that enhances an immune response to the part of the protein having the amino acid sequence represented by the formula X--Y.

36. A vaccine composition comprising a protein fused to a heterologous polypeptide, wherein the protein comprises the amino acid sequence represented by the formula: X--Y wherein X represents the N-terminal residue of the amino acid sequence from SEQ ID NO:2 and is any amino acid from position 1 to 69 of SEQ ID NO:2; wherein Y represents the C-terminal residue of the amino acid sequence from SEQ ID NO:2 and is any amino acid from position 452 to 651 of SEQ ID NO:2.

37. The vaccine composition according to claim 36 wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:2.

38. The vaccine composition according to claim 36 wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:4.

39. The vaccine composition according to claim 36 wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:6.

40. The vaccine composition according to claim 36 wherein the heterologous polypeptide has adjuvant activity.

41. The vaccine composition according to claim 40 wherein the heterologous polypeptide having adjuvant activity is suitable as an adjuvant for parenteral administration.

42. The vaccine composition according to claim 40 wherein the heterologous polypeptide having adjuvant activity is suitable as an adjuvant for mucosal administration.

43. The vaccine composition according to claim 42 wherein the heterologous polypeptide is subunit B of cholera toxin (CTB) or subunit B of E. coli heat-labile toxin (LTB).

44. The vaccine composition according to claim 40 wherein the heterologous polypeptide is a strong T-cell epitope and/or a strong B-cell epitope.

45. The vaccine composition according to claim 44 wherein the heterologous polypeptide is a strong T-cell epitope and/or a strong B-cell epitope from hepatitis B virus core antigen.

46. The vaccine composition according to claim 40 further comprising a compound that facilitates delivery and/or enhance an immune response to the part of the protein having the amino acid sequence represented by the formula X--Y.

47. The vaccine composition according to claim 46 wherein the compound that facilitates delivery and/or enhance the immune response is a liposome.

48. The vaccine composition according to claim 40 further comprising an adjuvant.

49. The vaccine composition according to claim 40 in unit dosage form.

50. A method for treating or preventing a Chlamydia infection, the

method comprising the step of administering to a subject the vaccine composition as defined in claim 1.

51. A method for treating or preventing a Chlamydia infection, the method comprising the step of administering to a subject the vaccine composition as defined in claim 19.

52. A method for treating or preventing a Chlamydia infection, the method comprising the step of administering to a subject the vaccine composition as defined in claim 36.

53. A method for treating or preventing a Chlamydia infection, the method comprising the step of administering to a subject the vaccine composition as defined in claim 13, and further comprising the step of administering to the subject an antibiotic, an antacid, sucralfate, a cytokine immunomodulator or a combination thereof.

54. The method according to claim 53 wherein the antibiotic is a macrolide, a tetracycline, or a derivative thereof.

55. The method according to claim 53 wherein the antibiotic is azithromycin or doxicyclin, and wherein the immunomodulator is interleukin-2 (IL-2), interleukin-12 (IL-12), or a steroid.

56. A vaccination kit comprising the vaccine composition as defined in claim 1 and instructions for its use in vaccinating a subject against Chlamydia infection.

57. A vaccination kit comprising the vaccine composition as defined in claim 19 and instructions for its use in vaccinating a subject against Chlamydia infection.

58. A vaccination kit comprising the vaccine composition as defined in claim 36 and instructions for its use in vaccinating a subject against Chlamydia infection.

59. A vaccination kit comprising the vaccine composition as defined in claim 13, at least one compound selected from the group consisting of an antibiotic, an antacid, sucralfate, a cytokine immunomodulator, and instructions for using the composition and compound in vaccinating a subject against Chlamydia infection.

60. The kit according to claim 59 wherein the antibiotic is a macrolide, a tetracycline, or a derivative thereof.

61. The kit according to claim 59 wherein the antibiotic is azithromycin or doxicyclin, and wherein the immunomodulator is interleukin-2 (IL-2), interleukin-12 (IL-12), or a steroid.

L22 ANSWER 6 OF 17 USPATFULL on STN
2005:214594 Chlamydia antigens and corresponding DNA fragments and uses thereof

Murdin, Andrew D., Newmarket, CANADA
Oomen, Raymond P., Aurora, CANADA
Wang, Joe, Etobicoke, CANADA
Dunn, Pamela, Mississauga, CANADA
Aventis Pasteur Limited (non-U.S. corporation)
US 2005186266 A1 20050825
APPLICATION: US 2005-107823 A1 20050418 (11)
PRIORITY: US 1999-123966P 19990312 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A vaccine composition comprising a protein and a compound, wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:2, and wherein the compound facilitates delivery and/or enhance an immune response to the part of the protein having the amino acid sequence set forth in SEQ ID NO:2.

2. The composition according to claim 1 wherein the protein is a fusion protein comprising the amino acid sequence set forth in SEQ ID NO:2 fused with a heterologous polypeptide.

3. The composition according to claim 1 wherein the heterologous polypeptide is a peptide tail for purifying the protein.

4. The vaccine composition according to claim 1 wherein the compound is a liposome.

5. The composition according to claim 4 wherein the liposome is at least one liposome selected from the group consisting of neutral liposomes, anionic liposomes, microspheres, ISCOMS, and **virus-like-particles**

6. The composition according to claim 1 wherein the compound is an adjuvant.

7. The composition according to claim 6 which is suitable for parenteral administration.

8. The composition according to claim 7 wherein the adjuvant is at least one adjuvant selected from the group consisting of an aluminum compound, RIBI, polyphosphazene, DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol and QS-21.

9. The composition according to claim 8 wherein the adjuvant is aluminum hydroxide, aluminum phosphate, or aluminum hydroxy phosphate.

10. The composition according to claim 6 which is suitable for mucosal administration.

11. The composition according to claim 10 wherein the adjuvant is at least one adjuvant selected from the group consisting of bacterial toxin, bacterial monophosphoryl lipid A (MPLA), saponin, polylactide glycolide (PLGA) microsphere, polyphosphazene, DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl)cholesterol, and QS-21.

12. The composition according to claim 11 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of cholera toxin (CT), E. coli heat-labile toxin (LT), Clostridium difficile toxin A, pertussis toxin (PT), and combinations, subunits, toxoids, or mutants thereof that retain adjuvant activity and/or have reduced toxicity.

13. The composition according to claim 12 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of native cholera toxin subunit B (CTB), Arg-7-Lys CT mutant, Arg-192-Gly LT mutant, Arg-9-Lys PT mutant, Glu-129-Gly PT mutant, Ser-63-Lys LT mutant, Ala-69-Gly LT mutant, Glu-110-Asp LT mutant, and Glu-112-Asp LT mutant.

14. The composition according to claim 11 wherein the adjuvant is bacterial monophosphoryl lipid A (MPLA) of E. coli, Salmonella minnesota, Salmonella typhimurium, or Shigella flexneri.

15. The composition according to claim 1 in unit dosage form.

16. A vaccine composition comprising a protein in unit dosage form, wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:2.

17. The composition according to claim 16, further comprising a compound that facilitates delivery and/or enhance an immune response to the part of the protein having the amino acid sequence set forth in SEQ ID NO:2.

18. The composition according to claim 17 wherein the compound is a liposome.

19. The composition according to claim 18 wherein the liposome is at least one liposome selected from the group consisting of neutral liposomes, anionic liposomes, microspheres, ISCOMS, and **virus-like-particles (VLPs)**.

20. The composition according to claim 16, further comprising an adjuvant.

21. The composition according to claim 20 which is suitable for parenteral administration.

22. The composition according to claim 21 wherein the adjuvant is at least one adjuvant selected from the group consisting of an aluminum compound, RIBI, polyphosphazene, DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol and QS-21.

23. The composition according to claim 22 wherein the adjuvant is aluminum hydroxide, aluminum phosphate, or aluminum hydroxy phosphate.

24. The composition according to claim 23 which is suitable for mucosal administration.

25. The composition according to claim 24 wherein the adjuvant is at least one adjuvant selected from the group consisting of bacterial toxin, bacterial monophosphoryl lipid A (MPLA), saponin, polylactide glycolide (PLGA) microsphere, polyphosphazene, DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl)cholesterol, and QS-21.

26. The composition according to claim 25 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of cholera toxin (CT), E. coli heat-labile toxin (LT), Clostridium difficile toxin A, pertussis toxin (PT), and combinations, subunits, toxoids, or mutants thereof that retain adjuvant activity and/or have reduced toxicity.

27. The composition according to claim 26 wherein the adjuvant is at least one bacterial toxin selected from the group consisting of native cholera toxin subunit B (CTB), Arg-7-Lys CT mutant, Arg-192-Gly LT mutant, Arg-9-Lys PT mutant, Glu-129-Gly PT mutant, Ser-63-Lys LT mutant, Ala-69-Gly LT mutant, Glu-110-Asp LT mutant, and Glu-112-Asp LT mutant.

28. The composition according to claim 25 wherein the adjuvant is bacterial monophosphoryl lipid A (MPLA) of E. coli, Salmonella minnesota, Salmonella typhimurium, or Shigella flexneri.

29. The composition according to claim 16, further comprising an additional Chlamydia polypeptide that enhances an immune response to the part of the protein having the amino acid sequence set forth in SEQ ID NO:2.

30. A vaccine composition comprising a protein fused to a heterologous polypeptide having adjuvant activity, wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:2.

31. The vaccine composition according to claim 30 wherein the heterologous polypeptide having adjuvant activity is suitable as an adjuvant for parenteral administration.

32. The vaccine composition according to claim 30 wherein the heterologous polypeptide having adjuvant activity is suitable as an adjuvant for mucosal administration.

33. The vaccine composition according to claim 32 wherein the heterologous polypeptide is subunit B of cholera toxin (CTB) or subunit B of E. coli heat-labile toxin (LTB).

34. The vaccine composition according to claim 30 wherein the heterologous polypeptide is a strong T-cell epitope and/or a strong B-cell epitope.

35. The vaccine composition according to claim 34 wherein the heterologous polypeptide is a strong T-cell epitope and/or a strong B-cell epitope from hepatitis B virus core antigen.

36. The vaccine composition according to claim 30 further comprising a compound that facilitates delivery and/or enhance the immune response to the part of the protein having the amino acid sequence set forth in SEQ ID NO:2.

37. The vaccine composition according to claim 36 wherein the compound that facilitates delivery and/or enhance the immune response is a liposome.

38. The vaccine composition according to claim 30 further comprising an adjuvant.

39. The vaccine composition according to claim 30 in unit dosage form.

40. A method for treating or preventing a Chlamydia infection, the method comprising the step of administering to a subject the vaccine composition as defined in claim 1.

41. A method for treating or preventing a Chlamydia infection, the method comprising the step of administering to a subject the vaccine composition as defined in claim 16.

42. A method for treating or preventing a Chlamydia infection, the method comprising the step of administering to a subject the vaccine composition as defined in claim 30.

43. A method for treating or preventing a Chlamydia infection, the method comprising the step of administering to a subject the vaccine composition as defined in claim 10, and further comprising the step of administering to the subject an antibiotic, an antacid, sucralfate, a cytokine immunomodulator or a combination thereof.

44. The method according to claim 43 wherein the antibiotic is a macrolide, a tetracycline, or a derivative thereof.

45. The method according to claim 43 wherein the antibiotic is azithromycin or doxicyclin, and wherein the immunomodulator is

interleukin-2 (IL-2), interleukin-12 (IL-12), or a steroid.

46. A vaccination kit comprising the vaccine composition as defined in claim 1 and instructions for its use in vaccinating a subject against Chlamydia infection.

47. A vaccination kit comprising the vaccine composition as defined in claim 16 and instructions for its use in vaccinating a subject against Chlamydia infection.

48. A vaccination kit comprising the vaccine composition as defined in claim 30 and instructions for its use in vaccinating a subject against Chlamydia infection.

49. A vaccination kit comprising the vaccine composition as defined in claim 10, at least one compound selected from the group consisting of an antibiotic, an antacid, sucralfate, a cytokine immunomodulator, and instructions for using the composition and compound in vaccinating a subject against Chlamydia infection.

50. The kit according to claim 49 wherein the antibiotic is a macrolide, a tetracycline, or a derivative thereof.

51. The kit according to claim 49 wherein the antibiotic is azithromycin or doxicyclin, and wherein the immunomodulator is interleukin-2 (IL-2), interleukin-12 (IL-12), or a steroid.

L22 ANSWER 7 OF 17 USPATFULL on STN
2005:143828 Lipoparticles comprising proteins, methods of making, and using the same.
Doranz, Benjamin J., Narberth, PA, UNITED STATES
Willis, Sharon, Wayne, PA, UNITED STATES
Ross, Eric, Philadelphia, PA, UNITED STATES
Greene, Tiffani Anne, Cherry Hill, NJ, UNITED STATES
US 2005123563 A1 20050609
APPLICATION: US 2004-901399 A1 20040728 (10)
PRIORITY: US 2003-491477P 20030730 (60)
US 2003-491633P 20030730 (60)
US 2003-498755P 20030829 (60)
US 2003-502478P 20030912 (60)
US 2003-509677P 20031007 (60)
US 2003-509608P 20031007 (60)
US 2003-509575P 20031007 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A lipoparticle comprising a viral protein component and a cellular protein, wherein said viral protein component consists essentially of a viral structural protein.

2. A lipoparticle comprising a viral protein component and a cellular protein, wherein said cellular protein is an unmodified protein, and wherein said lipoparticle is reverse transcription incompetent.

3. The lipoparticle of claims 1 or 2 wherein said cellular protein comprises a multiple spanning membrane protein.

4. The lipoparticle of claims 1 or 2 wherein said cellular protein comprises a G protein coupled receptor.

5. The lipoparticle of claim 1, wherein said cellular protein is a non-membrane protein that has been modified to be capable of localizing to the membrane of a cell.

6. A lipoparticle comprising an unmodified viral structural protein and a cellular protein, provided that the only viral proteins in said lipoparticle are structural proteins.

7. The lipoparticle of claim 6, wherein the cellular protein is a multiple spanning membrane protein.

8. A lipoparticle comprising a viral structural protein and a native cellular protein, provided that the only viral proteins in said lipoparticle are structural proteins.

9. The lipoparticle of claim 8, wherein the cellular protein is a multiple-spanning membrane protein.

10. The lipoparticle of claims 1 or 6 wherein said viral structural protein is Gag.

11. The lipoparticle of claim 10 wherein said Gag is from murine

leukemia virus or Rous sarcoma virus.

12. A composition comprising an isolated lipoparticle of any of claims 1 or 6 attached to a biosensor surface.

13. A lipoparticle comprising a viral protein component, a cellular protein and a G-protein.

14. The lipoparticle of claim 13, wherein said G-protein is a modified G-protein.

15. The lipoparticle of claim 14, wherein said modified G-protein comprises a fusion protein.

16. The lipoparticle of claim 15, wherein said fusion protein comprises a fluorescent protein, a linker, a viral protein, membrane protein, a protease cleavage sequence, or combinations thereof.

17. The lipoparticle of claim 13, wherein said cellular protein is a GPCR.

18. The lipoparticle of claim 17 said GPCR is a modified GPCR.

19. The lipoparticle of claim 18, wherein said modified GPCR comprises a fusion protein.

20. The lipoparticle of claim 19, wherein said fusion protein comprises a fluorescent protein, a linker, a viral protein, membrane protein, a protease cleavage sequence, or combinations thereof.

21. The lipoparticle of claim 13 further comprising a GTP analog.

22. The lipoparticle of claim 21 wherein said GTP analog is a fluorescent GTP analog.

23. A method of identifying modulators of a GPCR comprising: a) contacting a lipoparticle comprising a GPCR and a G-protein with a test compound; and b) measuring GPCR activity.

24. A method for producing a lipoparticle comprising: a) contacting a cell with nucleic acid encoding an unmodified viral structural protein and a cellular protein; and b) culturing said cell under conditions resulting in production of said lipoparticle, provided that the only viral protein encoded by said nucleic acid is a structural protein.

25. The method of claim 24 wherein a first nucleic acid molecule encodes said unmodified viral structural protein and a second nucleic acid molecule encodes said cellular protein.

26. The method of claim 24 wherein said contacting comprises infecting a cell with a non-enveloped virus, wherein said non-enveloped virus comprises a nucleic acid encoding the viral structural protein and a cellular protein.

27. The method of claim 26 wherein said non-enveloped virus is an adenovirus.

28. The method of claim 24 wherein said contacting comprises infecting a cell with an alphavirus, wherein said alphavirus comprises a nucleic acid encoding the viral structural protein and a cellular protein.

29. The method of claim 28, wherein said alphavirus is Semliki Forest Virus

30. The method of claim 24 wherein said cell is a non-human cell and said cellular protein is a human protein.

31. The method of claim 24 wherein the viral structural protein is Gag.

32. A method for producing a lipoparticle comprising: a) contacting a cell having a membrane protein of interest with an adenovirus encoding at least a viral Gag protein; and b) culturing said cell under conditions resulting in production of said lipoparticle.

33. A method for producing a lipoparticle comprising: a) contacting a cell with an adenoviral vector encoding at least a viral Gag protein and a cellular protein or an adenoviral vector encoding at least a viral Gag protein and a nucleic acid encoding said cellular protein; and b) culturing said cell under conditions resulting in production of said lipoparticle.

34. The method of any of claims 24, 32, or 33 wherein said protein is a membrane protein.

35. A chimeric viral vector comprising adenoviral nucleic acid and retroviral nucleic acid, provided that said retroviral nucleic acid comprises a sequence encoding Gag, but does not comprise a sequence encoding the envelope, promoter, or packaging signal of the retrovirus.

36. A method of eliciting an immune response in a subject comprising administering the composition of claims 1 or 6 to said subject.

37. A method of assessing the binding interaction of a protein with a ligand, said method comprising contacting a lipoparticle according to any of claims 1 or 6 comprising said protein, wherein said lipoparticle is attached to a substrate, with a ligand of said protein; and detecting any change in said substrate compared with any change in an otherwise identical substrate wherein said lipoparticle is not contacted with said ligand; wherein detecting a change in said substrate wherein said lipoparticle is contacted with said ligand compared with said otherwise identical substrate wherein said lipoparticle is not contacted with said ligand assesses said binding interaction of said protein with said ligand.

38. A method of identifying potential ligands of a protein, said method comprising contacting a lipoparticle according to any of claims 1 or 6 comprising said protein, wherein said lipoparticle is attached to a substrate, with a test ligand and detecting any change in said substrate compared with any change in an otherwise identical substrate wherein said lipoparticle is not contacted with said ligand; wherein detecting a change in said substrate wherein said lipoparticle is contacted with said ligand compared with said otherwise identical substrate wherein said lipoparticle is not contacted with said ligand identifies a ligand.

39. A method of identifying a compound that affects binding between a ligand and a protein, said method comprising contacting said compound with said ligand; contacting said compound/ligand complex with a lipoparticle according to any of claims 1 or 6 comprising said protein, wherein said lipoparticle is attached to a substrate; and detecting any change in said substrate compared with any change in an otherwise identical substrate wherein said compound is not contacted with said ligand and said ligand is contacted with said lipoparticle; wherein detecting a change in said substrate when said compound is contacted with said ligand compared with said otherwise identical substrate wherein said ligand is contacted with said lipoparticle assesses said effect of said compound.

40. A method of detecting a ligand of a protein in a test sample, said method comprising contacting a lipoparticle according to any of claims 1 or 6 comprising said protein, wherein said lipoparticle is attached to a substrate with a test sample; and detecting any change in said substrate compared with any change in an otherwise identical substrate wherein said lipoparticle is not contacted with said test sample; wherein detecting a change in said substrate wherein said lipoparticle is contacted with said ligand compared with said otherwise identical substrate wherein said lipoparticle is not contacted with said test sample indicates the presence of said ligand in said test sample.

41. The method of claim 24, wherein said nucleic acid comprise an adenovirus, bacculovirus, vaccinia virus, herpes virus, or alphavirus vector.

42. An immunogen comprising a lipoparticle according to any of claims 1 or 6.

43. A method of eliciting an immune response to a protein, said method comprising the introduction of a lipoparticle to an animal, and said lipoparticle being produced using the method of any of the claims 24, 32, or 33.

44. A method of eliciting an immune response to a protein, said method comprising the introduction of a lipoparticle to an animal, and said lipoparticle being a lipoparticle according to any of claims 1 or 6.

45. A method of determining the structure of a membrane protein comprising: a) isolating a membrane protein from a lipoparticle containing said membrane protein; and b) determining said structure of said membrane protein; wherein said membrane protein is not a viral envelope protein.

46. A method of determining the structure of a protein comprising: a) isolating a protein from a lipoparticle containing said membrane protein; and b) determining said structure of said protein; wherein said protein comprises a Gag fusion protein.

47. A composition comprising an array of lipoparticles attached to a

surface.

48. The composition of claim 47, wherein said surface comprises an optical biosensor surface, an optical fiber, a microfluidic device, a microcantilever, an acoustive wave sensor, or electromagnetic sensor.

49. The composition of claim 47, wherein said surface measures surface plasmon resonance, colorimetric diffraction grating, chemiluminescence, deflection of light, acoustic waves, or fluorescence.

50. The composition of claim 47, wherein said lipoparticles comprise membrane proteins derived from a naturally occurring source.

51. The composition of claim 50, wherein said naturally occurring source is a primary cell, an organ, a stem cell, or a cell line.

52. The composition of claim 47 wherein said surface has been modified to bind lipoparticles.

53. The composition of claim 52 wherein said modification comprises a cationic film, alkane modification, gamma-aminopropylsilane, histidine, Ni+2, protein A, protein G, protein L, lectin, biotin, or avidin.

54. The composition of claim 47 wherein said lipoparticles have been modified to bind to the surface.

55. A method of detecting an infectious pathogen in a sample comprising the steps of: a) contacting the sample with an array of lipoparticles attached to a surface, wherein said array of lipoparticles comprises membrane proteins that interact with various infectious pathogens; and b) detecting an interaction with said array of lipoparticles; wherein said detection of said interaction indicates the presence of an infectious pathogen.

56. A method of determining the presence of a substance in a sample comprising the steps of: a) contacting the sample with an array of lipoparticles attached to a surface, wherein said lipoparticles comprise membrane proteins that interact with said substance; and b) detecting an interaction with said array of lipoparticles; wherein said detection of said interaction indicates the presence of said substance.

57. A method of identifying an inhibitor of a binding activity of a substance to a membrane protein comprising the steps of: a. contacting said substance with an array of lipoparticles comprising said membrane protein attached to a surface to which said substance normally binds, in the presence of a potential inhibitor; and b. detecting an interaction of said substance with said array; wherein if an interaction is detected, then said potential inhibitor does not inhibit said binding and if an interaction is not detected then said potential inhibitor inhibits said binding.

58. The method of claim 57, wherein said potential inhibitor comprises a fluorescent tag, enzymatic tag, biotinylated tag, paramagnetic tag, a radioactive tag, or a combination thereof.

59. The method of any of claims 56 or 57, wherein said substance is an organic compound, chemical, peptide, protein, antibody, virus, bacteria, toxin, a monoclonal antibody, a low-molecular weight organic compound or a combination thereof.

60. A method for spotting lipoparticles, viruses, or **virus-like particles** in an array format onto a surface comprising including in the spotting medium a preservative.

61. The method of claim 60, wherein said preservative is trehalose, glycerol, collagen, sucrose, gelatin, or combinations thereof.

62. A method of identifying a binding partner of a membrane protein comprising: a) contacting a surface coated with lipoparticles, viruses, or **virus-like particles** comprising said membrane protein with an array comprising potential binding partners; and b) detecting binding of potential binding partner to said membrane protein.

63. The method of claim 62 wherein said potential binding partners comprise an antibody, antibody fragment, peptide, polypeptide, or small organic molecule.

64. A lipoparticle comprising a viral protein component and a cellular protein, wherein said viral protein component consists essentially of a viral structural protein, wherein said cellular protein is an ion channel protein or transporter protein.

65. The lipoparticle of claim 64 wherein said viral structural protein

is unmodified.

66. The lipoparticle of claim 64, wherein said lipoparticle comprises a fluorescent dye.

67. The lipoparticle of claim 64, wherein said lipoparticle comprises at least one modification to detect ion channel protein function or transporter protein function

68. A method to determine membrane protein function in a lipoparticle, virus, or **virus-like particle** comprising a membrane protein, wherein said lipoparticle, virus, or **virus-like particle** further comprises a detectable agent, wherein measuring either an increase or decrease in the detectable agent is used to determine the membrane protein function.

69. The method of claim 68, wherein said membrane protein is an ion channel.

70. The method of claim 68, wherein said detectable agent is a fluorescent probe.

71. The method of claim 70, wherein said fluorescent probe is a voltage-sensitive fluorescent dye, an ion-sensitive fluorescent dye, fluorescent lipid, fluorescent amino acid, fluorescent nucleic acid, or a fluorescent protein.

72. The method of claim 68, wherein said increase or decrease in detectable signal indicates an activation of said protein.

73. The method of claim 68, wherein said detectable agent comprises at least two fluorescent probes that interact by resonance energy transfer to produce a distinct fluorescent signal, wherein said distinct signal is used to determine said function of said protein.

74. A method of identifying a stimulator of a membrane protein comprising: a) contacting a lipoparticle comprising said membrane protein and a detectable agent with a compound; and b) measuring any change in the detectable agent; wherein said change in the detectable agent is used to indicate that said compound is a stimulator.

75. A method of identifying an inhibitor of a known stimulator of an ion channel protein or a transporter protein within a lipoparticle, wherein said lipoparticle comprises an ion channel or transporter, comprising the steps of: a) contacting said lipoparticle with said stimulator; b) contacting said lipoparticle with a test compound; c) measuring the function of said ion channel protein or transporter protein.

76. A method of detecting changes in ion concentration in a location comprising: a) microinjecting lipoparticles comprising a membrane protein and a detectable agent to said location; and b) detecting changes in ion concentration by measuring said change in said detectable agent.

77. An immunogenic composition comprising a lipoparticle comprising a protein of interest and at least one immunostimulatory component.

78. The immunogenic composition of claim 77, wherein said at least one immunostimulatory component comprises DNA, adenovirus, adjuvant, or proteins.

79. A method of producing antibodies against a protein comprising: a) administering the immunogenic composition according to claim 77 comprising said protein to an animal; and b) isolating said antibodies.

80. A method of identifying a binding partner of a membrane protein comprising: a) contacting a lipoparticle, virus, or **virus-like particle** comprising said membrane protein with a library, wherein said library comprises more than one potential binding partner; b) detecting the binding of said binding partner to said membrane protein.

81. The method of claim 80 wherein said library is a phage display library or ribosome display library.

82. The method of claim 80 wherein said library comprises antibodies.

83. The method of claim 82 wherein said antibody is a monoclonal antibody, a polyclonal antibody, an affinity-purified polyclonal antibody, a Fab fragment derived from a monoclonal antibody, an immunoglobulin-fusion protein, a single-chain Fv, an Fc-fusion protein, peptide, or polypeptide.

84. A method of transfecting a protein into a cell comprising contacting

said cell with a lipoparticle comprising said protein.

85. A method of transfecting a protein into a cell comprising contacting said cell with a lipoparticle comprising a viral protein component and said protein, wherein said viral protein component consists essentially of a viral structural protein.

86. A method of correcting a protein defect in an individual comprising administering a cell transfected according to any of claims 84 or 85.

87. A particle comprising a fluorophore wherein said fluorophore changes fluorescence in response to pH, membrane potential, oxidation state, NO level, ion concentration, ATP concentration, protein interaction, or combinations thereof m in size and wherein said particle is less than 1 µm.

88. The particle of claim 87, wherein said particle is a lipoparticle.

89. The particle of claim 87, wherein said particle is a virus particle or **virus-like particle**.

90. The particle of claim 87, wherein said fluorophore is a fluorescent protein or fluorescent dye.

91. The particle of claim 90 wherein said fluorescent protein comprises a fusion protein comprising Gag.

92. The particle of claim 90 wherein said fluorescent protein is a membrane protein or membrane protein fusion protein.

93. A lipoparticle comprising a Gag fusion protein and exogenous membrane protein, wherein said Gag fusion protein comprises a fluorescent protein or an enzymatic protein.

94. The lipoparticle of claim 93 wherein said membrane protein comprises a binding domain.

95. The lipoparticle of claim 94 wherein said binding domain comprises the ligand-binding portion of a cellular membrane protein.

96. The lipoparticle of claim 94 wherein said binding domain comprises an antibody-binding domain.

97. The lipoparticle of claim 96 wherein said antibody-binding domain is from Protein A, Protein G, or Protein L.

98. The lipoparticle of claim 97 further comprising one or more antibody-like molecules bound to said binding domain.

99. The lipoparticle of claim 98 wherein said antibody-like molecules comprises a monoclonal antibody, a polyclonal antibody, an affinity-purified polyclonal antibody, a Fab fragment derived from a monoclonal antibody, an immunoglobulin-fusion protein, a single chain Fv, an Fc-fusion protein, or combinations thereof.

100. The lipoparticle of claim 93 wherein said exogenous membrane protein comprises a reporter fusion protein.

101. A lipoparticle comprising a modified lipid.

102. The lipoparticle of claim 101 wherein said modified lipid comprises a biotin-coupled or a fluorescently-coupled lipid.

103. A lipoparticle comprising at least one of a radioactive molecule, a magnetic substance, a paramagnetic substance, a biotinylated molecule, an avidin-like molecule, gold, or combinations thereof and optionally a fluorophore.

104. A method of incorporating a molecule into a lipoparticle, virus or a **virus-like particle** comprising contacting an AM-ester form of said molecule with a lipoparticle comprising an esterase.

105. A method of incorporating a molecule into a lipoparticle, virus or a **virus-like particle** comprising contacting a soluble form of said molecule with said lipoparticle and performing electroporation, sonication, or vortexing.

106. A method of inducing pores in a lipoparticle comprising incubating said lipoparticle with a pore-forming peptide, an alkane, or a detergent.

107. A method of attaching a molecule to a lipoparticle, virus, or **virus-like particle** comprising contacting a modified molecule with

said lipoparticle, virus, or **virus-like particle**, wherein said lipoparticle, virus, or **virus-like particle** is able to bind to said modified molecule.

108. The method of claim 107 wherein said modified molecule comprises a biotin group or avidin group.

109. The method of claim 107 wherein said modified molecule comprises a lectin binding molecule.

110. A method of determining binding of a compound to a lipoparticle, virus, or **virus-like particle** comprising a) contacting said compound with said lipoparticle; and b) determining if said compound binds to said lipoparticle, wherein said compound or said compound and said lipoparticle comprises a fluorescent label.

111. The method of claim 110 wherein said determining comprises using a microscope, VELISA, flow cytometry, AVELISA, or immunofluorescence.

112. The method of claim 110 wherein said lipoparticle is attached to a bead.

113. The method of claim 110, wherein said compound is a ligand, a peptide, a protein, an antibody, an organic chemical compound, or an inorganic chemical compound.

114. A method of detecting the presence of an antigen in a sample comprising: a) contacting a lipoparticle comprising a binding partner for said antigen with said sample; and b) detecting a signal in said sample; wherein said detection of said signal indicates the presence of said antigen.

115. The method of claim 114 wherein said lipoparticle comprises an antibody-binding domain.

116. The method of claim 114 wherein said method is used to identify a pathogen, a disease diagnosis, or a drug.

117. A method of hybridizing an oligonucleotide to a target sequence in a lipoparticle, virus, or **virus-like particle** comprising contacting said oligonucleotide with said lipoparticle, virus, or **virus-like particle** comprising said target sequence under conditions that permit hybridization of said oligonucleotide to said target sequence.

118. The method of claim 117 wherein said target sequence is specific for a virus or virus family.

119. A method of detecting lipoparticle fusion comprising: a) contacting a lipoparticle, virus, or **virus-like particle** containing a fusigenic membrane protein with a lipoparticle comprising a receptor for said fusigenic membrane protein; and b) detecting said fusion; wherein said lipoparticle, virus, or **virus-like particle** comprises at least one reporter that is detectable upon fusion.

120. The method of claim 119 wherein said detection comprises detection by FRET, enzymatic activity, AM-ester cleavage, quenching, dequenching, or pyrene eximer formation.

121. A lipoparticle, virus, or **virus-like particle** attached to a bead, wherein said lipoparticle is attached to said bead via WGA, PEI, avidin-biotin interaction, poly-lysine interaction, or covalent coupling.

122. The lipoparticle of claim 121 wherein said lipoparticle comprises a first fluorescent label and said bead comprises a second fluorescent label.

123. A method for calculating the number of lipoparticles, viruses, or **virus-like particles** in a sample comprising: a) labeling said particles with a fluorophore; b) detecting said labeled particles, and c) counting said particles.

124. A method for calculating the quantity of particles, wherein said particles are lipoparticles, viruses, or **virus-like particles** comprising: a) measuring a detectable properties of a particle sample; and b) determining said quantity of particles by a correlation of amount of said properties to an amount of said particles.

125. A method for detecting the structural integrity of a membrane protein within a particle comprising a) contacting said particle with a molecule that binds to said membrane protein; and b) detecting binding of said molecule to said particle; wherein binding of said molecule to

said particle is indicative that the structural integrity of said membrane protein is intact.

126. The method of claim 125 wherein said molecule is an antibody, conformation-dependent antibody, ligand, agonist, toxin, or antagonist.

127. The method of claim 125 wherein said membrane protein is an ion channel, transporter protein, or a GPCR.

128. A method for determining the purity of a particle, wherein said particle is a lipoparticle, virus, or **virus-like particle** preparation comprising: a) quantifying number of particles in said preparation; b) quantifying total protein concentration in said preparation; and c) determining said purity by dividing the total protein concentration by the number of particles; and d) dividing the number obtained from step c) by the theoretical protein weight of said particle, wherein a value of about 1 is indicative of a pure sample and a value greater than 1 is indicative of a sample that is not completely pure.

129. A lipoparticle comprising at least one fusion protein, wherein said fusion protein comprises at least one binding domain, at least one transmembrane domain, and at least one reporter domain.

130. The lipoparticle of claim 129, wherein said binding domain comprises an antibody-binding domain.

131. The lipoparticle of claim 129 wherein said antibody-binding domain is an antibody-binding domain from Protein A, Protein G, or Protein L.

132. The lipoparticle of claim 129 wherein said binding domain comprises a ligand-binding portion of a cellular membrane protein

133. The lipoparticle of claim 129 wherein said reporter domain comprises a fluorescent protein.

134. The lipoparticle of claim 129 wherein said reporter domain comprises an amino acid sequence comprising enzymatic activity.

135. The lipoparticle of claim 129 wherein said reporter domain comprises an amino acid sequence comprising an inactive form of a protein that is made functional when brought into close proximity with a complementary inactive form.

136. The lipoparticle of claim 135, wherein said reporter comprises a fluorescent protein or an enzyme.

137. The lipoparticle of claim 129, wherein said lipoparticle comprises a first fusion protein and a second fusion protein, wherein each fusion protein comprises at least one binding domain, at least one transmembrane domain, and at least one reporter domain.

138. The lipoparticle of claim 137, wherein said first and second fusion proteins comprise two different reporter domains.

139. The lipoparticle of claim 137, wherein each of said first and second fusion proteins comprise an antibody-binding domain and wherein said lipoparticle further comprises one or more antibody-like molecules bound to said antibody-binding domain.

140. The lipoparticle of claim 139, wherein each of said antibody-like molecules comprises a monoclonal antibody, a polyclonal antibody, an affinity-purified polyclonal antibody, a Fab fragment derived from a monoclonal antibody, an immunoglobulin-fusion protein, a single-chain Fv, an Fc-fusion protein, or combinations thereof.

141. The lipoparticle of claim 139, wherein said first and second fusion proteins comprise two different antibody-like molecules that each recognizes different epitopes on the same protein.

142. The lipoparticle of claim 139, wherein said first and second fusion proteins comprise two different antibody-like molecules that each recognizes different epitopes on different proteins.

143. A method of detecting the presence of an antigen in a sample comprising contacting said sample with at least one lipoparticle comprising a binding partner wherein said particle comprises at least one fusion protein comprising at least one binding domain, at least one transmembrane domain, and at least one reporter domain and detecting the signal from said lipoparticle.

144. The method of claim 143 wherein said sample comprises at least one pathogenic protein, at least one whole-pathogenic organism, at least one

one tissue section, at least one cell, at least one antibody, or combinations thereof.

145. The method of claim 143, wherein said at least one lipoparticle comprises an array of lipoparticles.

146. The method of claim 143, wherein said antigen comprises an array of antigens.

147. A device comprising at least one lipoparticle and capable of being used to perform the method of claim 143.

L22 ANSWER 8 OF 17 USPATFULL on STN
2005:56171 Adjuvant viral particle.

Leclerc, Denis, Fossambault-sur-le-lac, CANADA
Majeau, Nathalie, Fossambault-sur-le-lac, CANADA
Tessier, Philippe, Cap-Rouge, CANADA
Lopez-Macias, Constantino III Roberto, Mexico D.F., MEXICO

US 2005048082 A1 20050303
APPLICATION: US 2003-609417 A1 20030701 (10)
PRIORITY: US 2002-393659P 20020705 (60)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:

1. An immunogen-carrier complex having an immupotentiation property, consisting of a viral-like particle (**VLP**) carrying at least one immunogen in fusion with a protein or fragment thereof of said **VLP**.

2. The immunogen-carrier complex of claim 1, wherein said immupotentiation property is at least one of an adjuvant effect, a capacity to enhance cell-mediated dependent antibody production or T-cell dependent antibody production, or the capacity of enhancing the expression of at least one costimulator on magrophages or other antigen presenting cells.

3. The immunogen-carrier complex of claim 1, wherein said immunogen is selected from the group consisting of a peptide, a protein, an hapten, and an allergen.

4. The immunogen-carrier complex of claim 1, wherein said immunogen is a viral, a bacterial, or a parasitical protein or fraction thereof capable of inducing an immune response in a human or an animal.

5. The immunogen-carrier complex of claim 1, wherein said immunogen is in carboxy- or amino-terminal fusion with a capsid, coque, or membrane protein of said **VLP**.

6. The immunogen-carrier of claim 1, wherein said **VLP** is a virus, a virus particle, a virion or a particle derived from assembly of a viral coat protein.

7. The immunogen-carrier complex of claim 1, wherein said **VLP** is selected from the group consisting or a plant potexvirus.

8. The immunogen-carrier complex of claim 7, wherein said potexvirus is a papaya mosaic virus.

9. The immunogen-carrier complex of claim 1, wherein said immunogen is in fusion at the outer surface of said **VLP**.

10. The immunogen-carrier complex of claim 1, wherein said immunogen is composed of one or more antigen domain, each antigen domain triggering a specific immune response.

11. The immunogen-carrier complex of claim 1, wherein said **VLP** is carrying immunogens having more than one specificity.

12. A method for immunopotentiating an immune response in a human or an animal which comprises administering to said human or animal an immunogen-carrier consisting of a viral-like particle (**VLP**) carrying at least one immunogen in fusion with a protein or fragment thereof of said **VLP**, or administering a **VLP** or a fragment thereof concomitantly with an antigen not directly linked to said **VLP**.

13. A polynucleotide encoding a immunogen-carrier complex consisting of a viral-like particle (**VLP**) carrying at least one immunogen in fusion with a protein or A polynucleotide encoding a immunogen-carrier complex consisting of a fragment thereof of said **VLP**, or a **VLP** alone, said immunogen-carrier complex having the capacity of being assembled when expressed in a plant cell, an animal cell or a microorganism.

14. Use of a VLP or an immunogen-carrier complex consisting of a viral-like particle (**VLP**) carrying at least one immunogen in fusion with a protein or fragment thereof of said **VLP** in the preparation of a composition for inducing an immune response against said protein or fragment thereof.

15. A composition for immunopotentiating an immune response against an antigen comprising **VLP** or an immunogen-carrier complex consisting of a viral-like particle (**VLP**) carrying at least one immunogen in fusion with a protein or fragment thereof of said **VLP**.

16. An immunopotentiator comprising **VLP** or a fragment thereof.

17. A composition comprising a viral-like particle (**VLP**) and a protein or an extract derived from a virus, bacteria or parasite.

18. The composition according to claim 17, for use as a vaccine.

19. Use of a papaya mosaic virus as an adjuvant.

L22 ANSWER 9 OF 17 USPATFULL on STN
2005:54512 Methods for identifying a compound that can bind to membrane-bound receptors.
Botfield, Martyn C., Boston, MA, UNITED STATES
Peng, Jeffrey Weilee, Granger, IN, UNITED STATES
US 2005046421 A1 20050303
APPLICATION: US 2004-837558 A1 20040430 (10)
PRIORITY: US 2001-340946P 20011030 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method of identifying a compound that can bind to a membrane-bound receptor comprising the steps of: a) providing Nuclear Overhauser Effect NMR data or data derived therefrom on a complex of a receptor bound to a known ligand for said receptor, wherein said receptor is incorporated into a **virus-like particle**; b) employing said data together with computational means to identify a compound capable of binding to said receptor through intermolecular interactions identified between said receptor and said known ligand of the receptor using the data provided in step a); c) contacting said compound with said receptor under conditions that allow said compound to bind to said receptor; and d) determining whether said compound binds to said receptor.

2. The method according to claim 1, wherein in step a), the data provided is structural coordinate data derived from said Nuclear Overhauser Effect NMR data.

3. A method of identifying intermolecular interactions between a receptor and a known ligand of the receptor comprising the steps of: a) contacting said receptor incorporated into a **virus-like particle** with said known ligand of the receptor under conditions that allow the formation of a complex between said ligand and said receptor; b) subjecting said complex to NMR to generate Nuclear Overhauser Effect data; c) optionally converting said Nuclear Overhauser Effect data to structural coordinate data or to bond angle data; and d) interpreting said Nuclear Overhauser Effect data or said optionally converted Nuclear Overhauser Effect data so as to identify intermolecular interactions between the receptor and the ligand in said complex.

4. A computer for producing a three-dimensional representation of a compound bound to a receptor comprising: a) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said data comprises Nuclear Overhauser Effect data or data derived therefrom obtained by subjecting a complex comprising a receptor incorporated into a **virus-like particle** and a compound bound to said receptor to NMR; b) a working memory for storing instructions for processing said machine-readable data; c) a central-processing unit coupled to said working memory and to said machine-readable data storage medium for processing said machine readable data into said three-dimensional representation; and d) a display coupled to said central-processing unit for displaying said three-dimensional representation.

5. A method for evaluating the potential of a chemical entity to associate with a membrane-bound receptor comprising the steps of: a) employing computational means to perform a fitting operation between the chemical entity and the receptor utilizing Nuclear Overhauser Effect NMR data or data derived therefrom obtained from a complex comprising a receptor incorporated into a **virus-like particle** and a compound bound to said receptor; and b) analyzing the results of said fitting operation to quantify the association between the chemical entity and

the receptor.

6. The method according to claim 5, comprising the additional steps of: c) repeating steps a) and b) on a series of different chemical entities; d) selecting two or more chemical entities that: i) are determined to have the potential to bind to the receptor based upon the analysis of the fitting operations; and ii) are capable of being chemically bound to one another directly or through a linker moiety; e) contacting a compound that comprises the chemical entities selected in step d) chemically bound to one another directly or through a linker moiety with said receptor under conditions that allow said compound to bind to said receptor; and f) determining whether said compound binds to said receptor.

L22 ANSWER 10 OF 17 USPATFULL on STN
2004:30662 Isolated amphiphilic peptides derived from the cytoplasmic tail of viral envelope proteins.
Rozenberg, Yanina, Studio City, CA, UNITED STATES
Anderson, W. French, San Marino, CA, UNITED STATES
US 2004022799 A1 20040205
APPLICATION: US 2002-304494 A1 20021125 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An isolated peptide comprising a fragment of a viral envelope protein, wherein said peptide is free of the portion of the envelope protein N-terminal of the membrane-spanning region of the envelope protein, said peptide having a membrane-destabilizing activity.

2. The peptide of claim 1 wherein the membrane-destabilizing activity of said peptide is sufficient to induce an electrophysiologically detectable increase of the release of a suitable marker from a liposome at an active concentration of 30 mM peptide/1 mol lipid in a suitable assay.

3. The peptide of claim 2, wherein said peptide forms an α-helical amphiphilic structure.

4. The peptide of claim 3 having a hydrophobic moment μ of at least 0.9 as calculated using DNASIS software employing the Chou, Fasman and Rose algorithm and calculated with the Kyte and Doolittle algorithm.

5. The peptide of claim 4 wherein said fragment comprises at least 8 amino acids.

6. The peptide of claim 5 wherein said fragment comprises at least the first 8 amino acids of the N-terminal portion of the cytoplasmic tail region of the envelope protein.

7. The peptide of claim 6 wherein said fragment comprises at least one out-of-phase residue.

8. The peptide of claim 7 wherein a portion of said peptide is present in said membrane-spanning region of said viral envelope protein.

9. The peptide of claim 8 wherein said peptide comprises the amino acid sequence of SEQ ID NO:1.

10. Derivatives and analogues of the peptide of claim 1 having at least one substitution of an amino acid residue that maintains the membrane-destabilizing activity of said peptide and/or having the reverse sequence of said peptide.

11. Use of the peptide of claim 1, or a nucleic acid encoding said peptide, for the preparation of a viral or synthetic vector.

12. Use of the peptide of claim 1, or a nucleic acid encoding said peptide, for the preparation of a medicament.

13. Use of an amphiphilic compound having a membrane-destabilizing activity for the preparation of a viral vector.

14. A peptide selected from the group consisting of (SEQ ID NO:2) and (SEQ ID NO:3) and derivatives and analogues of (SEQ ID NO:2) and (SEQ ID NO:3) having at least one amino acid substitution of (SEQ ID NO:2) and (SEQ ID NO:3) that maintains the membrane-destabilizing activity of said peptide.

15. The peptide of claim 14 wherein said peptide is (SEQ ID NO:2).

16. The peptide of claim 14 wherein said peptide is (SEQ ID NO:3).

17. A viral particle including a modified envelope protein, wherein said modified envelope protein includes the peptide of claim 1, wherein said peptide is located in a portion of said envelope protein external to the viral membrane.

18. The viral particle of claim 17 wherein said modified envelope protein further includes a targeting polypeptide including a binding region that binds to a liqand.

19. A **retroviral vector particle** including a retroviral envelope protein and the peptide of claim 1, wherein said peptide is attached to the retroviral membrane.

20. The **retroviral vector particle** of claim 19 wherein said retroviral envelope protein is a modified envelope protein that includes a targeting polypeptide that binds to a ligand.

21. A **retroviral vector particle** including a retroviral envelope protein, a targeting polypeptide including a binding region that binds to a ligand, and the peptide of claim 1, wherein each of said targeting polypeptide and the peptide of claim 1 is attached to the retroviral membrane.

22. A **retroviral vector particle** including a retroviral envelope protein, and a polypeptide including a targeting polypeptide including a binding region that binds to a ligand, a spacer moiety, and the peptide of claim 1, wherein said polypeptide is attached to the retroviral membrane.

23. A **retroviral vector particle** including (i) a targeting polypeptide including a binding region that binds to a ligand and (ii) the peptide of claim 1, wherein each of said targeting polypeptide and the peptide of claim 1 is attached separately to the membrane of said **retroviral vector particle,** and said **retroviral vector particle** does not include a retroviral envelope protein.

24. A **retroviral vector particle** including a polypeptide including (i) a targeting polypeptide including a binding region that binds to a ligand, (ii) a spacer moiety, and (iii) the peptide of claim 1, wherein said polypeptide is attached to the membrane of said **retroviral vector particle,** and said **retroviral vector particle** does not include a retroviral envelope protein.

25. The viral particle of claim 17 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

26. The **retroviral vector particle** of claim 19 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

27. The **retroviral vector particle** of claim 21 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

28. The **retroviral vector particle** of claim 22 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

29. The **retroviral vector particle** of claim 23 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

30. The **retroviral vector particle** of claim 24 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

31. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the viral particle of claim 25.

32. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the **retroviral vector particle** of claim 26.

33. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the **retroviral vector particle** of claim 27.

34. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the **retroviral vector particle** of claim 28.

35. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the **retroviral vector particle** of claim 29.

36. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the **retroviral vector particle** of claim 30.

37. A packaging cell including a polynucleotide encoding the retroviral gag protein, a polynucleotide encoding the retroviral pol protein, and a polynucleotide encoding a viral envelope protein including the peptide of claim 1.

38. The cell of claim 37 wherein said viral envelope protein further includes a targeting polypeptide including a binding region that binds to a ligand.

39. A producer cell formed from the packaging cell of claim 37.

40. A producer cell formed from the packaging cell of claim 38.

41. A packaging cell including a polynucleotide encoding the retroviral gag protein, a polynucleotide encoding the retroviral pol protein, a polynucleotide encoding the retroviral env protein, a polynucleotide including a nucleic acid sequence encoding the peptide of claim 1 and a nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein, and a polynucleotide including a nucleic acid sequence encoding a targeting polypeptide including a binding region which binds to a ligand and a nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein.

42. A producer cell formed from the packaging cell of claim 41.

43. A packaging cell including a polynucleotide encoding the retroviral gag protein, a polynucleotide encoding the retroviral pol protein, a polynucleotide encoding the retroviral env protein, and a polynucleotide including a first nucleic acid sequence encoding the peptide of claim 1, a second nucleic acid sequence encoding a spacer moiety, a third nucleic acid sequence encoding a targeting polypeptide including a binding region that binds to a ligand, and a fourth nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein.

44. A producer cell formed from the packaging cell of claim 43.

45. A pre-packaging cell including a polynucleotide encoding the retroviral gag protein, a polynucleotide encoding the retroviral pol protein, a polynucleotide including a nucleic acid sequence encoding the peptide of claim 1 and a nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein, and a polynucleotide including a nucleic acid sequence encoding a targeting polypeptide including a binding region that binds to a ligand and a nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein.

46. A pre-packaging cell line including a polynucleotide encoding the retroviral gag protein, a polynucleotide encoding the retroviral pol protein, and a polynucleotide including (i) a first nucleic acid sequence encoding the peptide of claim 1, (ii) a second nucleic acid sequence encoding a spacer moiety, (iii) a third nucleic acid sequence encoding a targeting polypeptide including a binding region that binds to a ligand, and (iv) a fourth nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein.

L22 ANSWER 11 OF 17 USPATFULL on STN
2003:334942 Immunogenic peptides, and method of identifying same.
Katritch, Vsevolod, San Diego, CA, UNITED STATES
Bordner, Andrew, San Diego, CA, UNITED STATES
Deans, Robert, Claremont, CA, UNITED STATES
Sumner, Mary, San Diego, CA, UNITED STATES
US 2003235818 A1 20031225
APPLICATION: US 2003-410647 A1 20030408 (10)
PRIORITY: US 2002-371250P 20020408 (60)
US 2002-371256P 20020408 (60)
US 2002-373668P 20020417 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for identifying an immunogenic peptide representative of a structural element of a target protein having a known three dimensional structure, comprising: selecting epitopes of the target protein based on the three dimensional structure of the target protein, thereby obtaining selected epitopes; detecting, in a molecular model of a polypeptide comprising a selected epitope linked to a scaffold protein, an epitope having a three dimensional conformation corresponding to the three dimensional structure of the epitope in the target protein, thereby identifying a candidate immunogenic peptide representative of a structural element of the target protein; and detecting that antibodies induced by the candidate immunogenic peptide selectively bind to the target protein, thereby identifying an immunogenic peptide

representative of a structural element of the target protein.

2. The method of claim 1, wherein the scaffold protein comprises a viral coat protein.

3. The method of claim 2, wherein the viral coat protein comprises a Hepatitis B core protein.

4. The method of claim 1, wherein the selected epitope is linked to the scaffold protein via a linker moiety.

5. The method of claim 4, wherein the linker moiety comprises a peptide.

6. The method of claim 1, wherein the polypeptide comprising a selected epitope linked to a scaffold protein is a fusion protein.

7. The method of claim 1, wherein the scaffold protein comprises a poly(amino acid).

8. The method of claim 7, wherein the poly(amino acid) comprises poly(lysine).

9. The method of claim 1, wherein the target protein comprises a protein of an infectious microorganism.

10. The method of claim 9, wherein the infectious microorganism is a bacterium.

11. The method of claim 10, wherein the infectious microorganism causes anthrax.

12. The method of claim 11, wherein the target protein comprises anthrax protective antigen.

13. The method of claim 11, wherein the target protein comprises anthrax lethal factor.

14. The method of claim 2, further comprising assembling a plurality of immunogenic peptides, each immunogenic peptide comprising an epitope linked to a viral coat protein, into a **virus-like particle**.

15. The method of claim 14, wherein epitopes of the immunogenic peptides of the plurality are the same.

16. The method of claim 14, wherein epitopes of the immunogenic peptides of the plurality comprise anthrax protective antigen epitopes and anthrax lethal factor epitopes.

17. An immunogenic peptide identified according to the method of claim 1.

18. An isolated peptide, consisting of: amino acid residues 606 to 705 of SEQ ID NO:30; amino acid residues 606 to 735 of SEQ ID NO:30; amino acid residues 606 to 706 of SEQ ID NO:30; amino acid residues 606 to 704 of SEQ ID NO:30; amino acid residues 606 to 734 of SEQ ID NO:30; amino acid residues 607 to 703 of SEQ ID NO:30; amino acid residues 606 to 732 of SEQ ID NO:30; amino acid residues 604 to 707 of SEQ ID NO:30; amino acid residues 606 to 730 of SEQ ID NO:30; or amino acid residues 606 to 733 of SEQ ID NO:30.

19. An isolated antibody that selectively binds a peptide of claim 18, provided said antibody does not substantially bind a peptide comprising: amino acid residues 596 to 735 of SEQ ID NO:30; amino acid residues 679 to 693 of SEQ ID NO:30; amino acid residues 703 to 722 of SEQ ID NO:30; or amino acid residues 671 to 721 of SEQ ID NO:30.

20. An isolated polynucleotide encoding the peptide of claim 18.

21. An isolated peptide, consisting of: amino acid residues 17 to 153 of SEQ ID NO:30; amino acid residues 261 to 454 of SEQ ID NO:30; or amino acid residues 487 to 594 of SEQ ID NO:30.

22. A composition, comprising at least a first peptide of claim 18 operatively linked to at least a first heterologous molecule.

23. The composition of claim 22, wherein the first heterologous molecule comprises a linker moiety.

24. The composition of claim 23, wherein the linker moiety comprises a peptide linker.

25. The composition of claim 24, wherein the peptide linker comprises an oligo(glycine) linker.

26. The composition of claim 24, wherein the peptide linker comprises a glycine-serine-alanine linker.

27. The composition of claim 22, wherein the first heterologous molecule comprises a heterologous peptide.

28. The composition of claim 27, which comprises a fusion protein.

29. The composition of claim 27, wherein the heterologous peptide comprises a scaffold protein.

30. The composition of claim 29, wherein the scaffold protein comprises a viral coat protein.

31. The composition of claim 30, wherein the viral coat protein comprises a Hepatitis B core protein.

32. The composition of claim 31, wherein the Hepatitis B core protein consists of amino acid residues 1 to 77 and amino acid residues 80 to 149 of SEQ ID NO:34, and wherein the peptide of claim B 1 is operatively linked to amino acid residue 77 and to amino acid residue 80 of SEQ ID NO:34.

33. The composition of claim 32, wherein the peptide of claim 18 is operatively linked to amino acid residue 77 via a first linker moiety and is operatively linked to amino acid residue 80 via a second linker moiety.

34. The method of claim 33, wherein the first linker moiety and the second linker moiety are the same.

35. The method of claim 33, wherein each of the first linker moiety and the second linker moiety is a peptide.

36. The composition of claim 22, further comprising at least a second heterologous molecule.

37. The composition of claim 36, wherein at least the first heterologous molecule comprises a linker moiety or a scaffold protein.

38. The composition of claim 37, wherein the first heterologous molecule comprises a linker moiety and the second heterologous molecule comprises a scaffold protein, and wherein the peptide of claim 18 is operatively linked to the scaffold protein via the linker moiety.

39. The composition of claim 36, comprising, in operative linkage, a first portion of a scaffold protein, a first peptide linker, the peptide of claim 18, a second peptide linker, and a second portion of the scaffold protein.

40. The composition of claim 39, wherein the scaffold protein comprises a viral coat protein.

41. The composition of claim 39, which comprises an amino acid sequence as set forth in SEQ ID NO:36.

42. The composition of claim 40, which comprises a plurality of polypeptides, wherein polypeptides of the plurality comprise a peptide of claim B 1 operatively linked to a viral coat protein.

43. The composition of claim 42, wherein polypeptides of the plurality are assembled to form a **virus-like particle**.

44. The composition of claim 22, wherein the first heterologous molecule comprises a tag.

45. The composition of claim 44, wherein the tag comprises a peptide tag.

46. The composition of claim 45, wherein the peptide tag comprises an oligo(histidine) tag.

47. The composition of claim 22, which is comprises immunogenic peptide representative of a structural element of the target protein.

48. The composition of claim 47, further comprising a carrier.

49. The composition of claim 48, wherein carrier comprises an adjuvant.

50. A polynucleotide encoding the composition of claim 22.

51. The polynucleotide of claim 50, which comprises SEQ ID NO:35.

52. The polynucleotide of claim 50, which is contained in a vector.

53. The polynucleotide of claim 52, wherein the vector is a viral vector.

54. The polynucleotide of claim 50, which is contained in a matrix.

55. The polynucleotide of claim 54, wherein the matrix comprises liposomes or microbubbles.

56. A method of stimulating an immune response in a subject, comprising administering the composition of claim 22, or a polynucleotide encoding the composition, to a subject under conditions suitable for stimulating an immune response.

57. The method of claim 56, wherein the immune response comprises a protective immune response.

58. The method of claim 56, wherein the subject is a mammalian subject.

59. The method of claim 56, wherein the subject is a human subject.

60. A method for identifying an immunogenic peptide representative of a structural element of a target protein having a known amino acid sequence but an unknown three dimensional structure, comprising: generating a molecular model of a three dimensional structure of the target protein based on the three dimensional structure of a homologous protein; selecting epitopes of the target protein based on the molecular model of the three dimensional structure of the target protein, thereby obtaining selected epitopes; detecting, in a molecular model of a chimeric polypeptide comprising at least one selected epitope having a constrained structure, an epitope having a three dimensional conformation corresponding to the molecular model of the three dimensional structure of the epitope in the target protein, thereby identifying a candidate immunogenic peptide representative of a structural element of the target protein; and detecting that antibodies induced by the candidate immunogenic peptide selectively bind to the target protein, thereby identifying an immunogenic peptide representative of a structural element of the target protein.

61. The method of claim 60, wherein the chimeric polypeptide comprises a plurality of linked selected epitopes.

62. The method of claim 61, wherein the selected epitopes of the plurality are the same.

63. The method of claim 60, wherein the chimeric polypeptide further comprises at least one linker peptide, which is linked to at least one selected epitope of the chimeric polypeptide.

64. The method of claim 60, wherein the constrained structure of the selected epitope is due to at least one disulfide bond in the chimeric polypeptide.

65. The method of claim 60, wherein the constrained structure of the selected epitope is due to cyclization of the chimeric polypeptide.

66. The method of claim 60, wherein the chimeric polypeptide further comprises a scaffold protein.

67. The method of claim 66, wherein the scaffold protein comprises a viral coat protein.

68. The method of claim 67, wherein the viral coat protein comprises a Hepatitis B core protein.

69. The method of claim 66, wherein the scaffold protein comprises a poly(amino acid).

70. The method of claim 69, wherein the poly(amino acid) comprises poly(lysine).

71. The method of claim 66, wherein the selected epitope is linked to the scaffold protein via a linker moiety.

72. The method of claim 71, wherein the linker moiety comprises a peptide.

73. The method of claim 60, wherein the target protein comprises a protein of an infectious microorganism.

74. The method of claim 73, wherein the infectious microorganism is a

bacterium.

75. The method of claim 74, wherein the target protein comprises an autotransporter.

76. The method of claim 75, wherein the autotransporter comprises an autotransporter as set forth in Table 1.

77. The method of claim 74, wherein the infectious microorganism is a Shigella species.

78. The method of claim 77, wherein the Shigella species is Shigella flexneri.

79. The method of claim 77, wherein the target protein comprises a Shigella protease involved in colonization (pic) gene product.

80. The method of claim 74, wherein the infectious microorganism is a Yersinia species.

81. The method of claim 80, wherein the Yersinia species is Yersinia pestis.

82. The method of claim 80, wherein the target protein comprises a Yersinia murine toxin (YMt).

83. The method of claim 67, further comprising assembling a plurality of immunogenic peptides, each immunogenic peptide comprising an epitope linked to a viral coat protein, into a **virus-like particle**.

84. The method of claim 83, wherein epitopes of the immunogenic peptides of the plurality are the same.

85. An immunogenic peptide identified according to the method of claim 60.

86. An isolated peptide, consisting of a peptide as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:1 1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, or SEQ ID NO:28.

87. An isolated polynucleotide encoding the peptide of claim 86.

88. The polynucleotide of claim 87, which is contained in a vector.

89. The polynucleotide of claim 88, wherein the vector is a viral vector.

90. The polynucleotide of claim 87, which is contained in a matrix.

91. The polynucleotide of claim 90, wherein the matrix comprises liposomes or microbubbles.

92. A composition, comprising at least a first peptide of claim 86 operatively linked to at least a first heterologous molecule.

93. The composition of claim 92, comprising a plurality of peptides of claim 86, wherein the peptides of the plurality are linked to each other.

94. The composition of claim 93, wherein peptides of the plurality are the same.

95. The composition of claim 92, wherein the first heterologous molecule comprises a linker moiety.

96. The composition of claim 95, wherein the linker moiety comprises a peptide linker.

97. The composition of claim 92, wherein the first heterologous molecule comprises a first heterologous peptide.

98. The composition of claim 97, wherein the first heterologous peptide comprises a scaffold protein.

99. The composition of claim 98, wherein the scaffold protein comprises a poly(amino acid).

100. The composition of claim 99, wherein the poly(amino acid) comprises poly(lysine).

101. The composition of claim 98, wherein the scaffold protein comprises a viral coat protein.

102. The composition of claim 101, wherein the viral coat protein comprises a Hepatitis B core protein.

103. The composition of claim 102, wherein the Hepatitis B core protein consists of amino acid residues 1 to 77 and amino acid residues 80 to 149 of SEQ ID NO:34, and wherein the peptide of claim 86 is operatively linked to amino acid residue 77 and to amino acid 80 of SEQ ID NO:34.

104. The composition of claim 101, comprising a plurality of peptides of claim 86, wherein each peptide of the plurality is operatively linked to a viral coat protein.

105. The composition of claim 104, which are assembled to form a **virus-like particle**.

106. The composition of claim 92, further comprising at least a second heterologous molecule.

107. The composition of claim 106, wherein at least the first heterologous molecule comprises a linker moiety or a scaffold protein.

108. The composition of claim 106, wherein the first heterologous molecule comprises a linker moiety and the second heterologous molecule comprises a scaffold protein, and wherein the linker moiety operatively links the peptide of claim NI to the scaffold protein.

109. The composition of claim 106, comprising, in operative linkage, a first portion of a scaffold protein, a first peptide linker, the peptide of claim Nl, a second peptide linker, and a second portion of the scaffold protein.

110. The composition of claim 109, wherein the scaffold protein comprises a viral coat protein.

111. The composition of claim 92, wherein the first heterologous molecule comprises a tag.

112. The composition of claim 92, wherein the first heterologous molecule comprises a carrier protein.

113. The composition of claim 92, which is an immunogenic composition.

114. The composition of claim 113, further comprising an adjuvant.

115. A method of stimulating an immune response in a subject, comprising administering the composition of claim 92, or a polynucleotide encoding the composition, to a subject under conditions suitable for stimulating an immune response.

116. The method of claim 115, wherein the immune response comprises a protective immune response.

117. The method of claim 115, wherein the subject is a mammalian subject.

118. The method of claim 115, wherein the subject is a human subject.

L22 ANSWER 12 OF 17 USPATFULL on STN
2003:318223 Expression of HIV polypeptides and production of **virus-like particles**.
Barnett, Susan, San Francisco, CA, UNITED STATES
Megede, Jan Zur, San Francisco, CA, UNITED STATES
Lian, Ying, Vallejo, CA, UNITED STATES
Hartog, Karin, Piedmont, CA, UNITED STATES
Liu, Hong, Castro Valley, CA, UNITED STATES
Greer, Catherine, Oakland, CA, UNITED STATES
Selby, Mark, Berkeley, CA, UNITED STATES
US 2003223964 A1 20031204
APPLICATION: US 2003-387336 A1 20030311 (10)
PRIORITY: US 1998-114495P 19981231 (60)
US 1999-168471P 19991201 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV Gag polypeptide, wherein the polynucleotide sequence encoding said Gag polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented

2. The expression cassette of claim 1, comprising, a polynucleotide sequence encoding a polypeptide including an HIV Gag polypeptide, wherein the polynucleotide sequence encoding said Gag polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:9.

3. The expression cassette of claim 1, wherein said polynucleotide sequence encoding a polypeptide including an HIV Gag polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:4.

4. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV protease polypeptide.

5. The expression cassette of claim 4,wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:78, and SEQ ID NO:79.

6. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV reverse transcriptase polypeptide.

7. The expression cassette of claim 6, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, and SEQ ID NO:84.

8. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV tat polypeptide.

9. The expression cassette of claim 8, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:87, SEQ ID NO:88, and SEQ ID NO:89.

10. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV polymerase polypeptide, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:6.

11. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV polymerase polypeptide, wherein (i) the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:4, and (ii) wherein the sequence is modified by deletions of coding regions corresponding to reverse transcriptase and integrase.

12. The expression cassette of claim 11, wherein said polynucleotide sequence preserves T-helper cell and CTL epitopes.

13. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HCV core polypeptide, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:7.

14. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV Env polypeptide, wherein the polynucleotide sequence encoding said Env polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:71 (FIG. 58) or SEQ ID NO:72 (FIG. 59).

15. The expression cassette of claim 14, wherein said Env polypeptide includes sequences flanking a V1 region but has a deletion in the V1 region itself.

16. The expression cassette of claim 15, wherein the polynucleotide sequence encoding the polypeptide comprises the sequence presented as SEQ ID NO:65 (FIG. 52 gp160.modUS4.delV1).

17. The expression cassette of claim 14, wherein said Env polypeptide includes sequences flanking a V2 region but has a deletion in the V2 region itself.

18. The expression cassette of claim 17, wherein the polynucleotide

sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:60 (FIG. 47); and SEQ ID NO:66 (FIG. 53).

19. The expression cassette of claim 17, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:34 (FIG. 20); SEQ ID NO:37 (FIG. 24); SEQ ID NO:40 (FIG. 27); SEQ ID NO:43 (FIG. 30); SEQ ID NO:46 (FIG. 33); SEQ ID NO:76 (FIG. 64) and SEQ ID NO:49 (FIG. 36).

20. The expression cassette of claim 14, wherein said Env polypeptide includes sequences flanking a V1/V2 region but has a deletion in the V1/V2 region itself.

21. The expression cassette of claim 20, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:59 (FIG. 46); SEQ ID NO:61 (FIG. 48); SEQ ID NO:67 (FIG. 54); and SEQ ID NO:75 (FIG. 63).

22. The expression cassette of claim 20, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:35 (FIG. 21); SEQ ID NO:38 (FIG. 25); SEQ ID NO:41 (FIG. 28); SEQ ID NO:44 (FIG. 31); SEQ ID NO:47 (FIG. 34) and SEQ ID NO:50 (FIG. 37).

23. The expression cassette of claim 14, wherein said Env polypeptide has a mutated cleavage site that prevents the cleavage of a gp140 polypeptide into a gp120 polypeptide and a gp41 polypeptide.

24. The expression cassette of claim 23, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:57 (FIG. 44); SEQ ID NO:61 (FIG. 48); and SEQ ID NO:63 (FIG. 50).

25. The expression cassette of claim 23, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:39 (FIG. 26); SEQ ID NO:40 (FIG. 27); SEQ ID NO:41 (FIG. 28); SEQ ID NO:42 (FIG. 29); SEQ ID NO:43 (FIG. 30); SEQ ID NO:44 (FIG. 31); SEQ ID NO:45 (FIG. 32); SEQ ID NO:46 (FIG. 33); and SEQ ID NO:47 (FIG. 34).

26. The expression cassette of claim 14, wherein said Env polypeptide includes a gp160 Env polypeptide or a polypeptide derived from a gp160 Env polypeptide.

27. The expression cassette of claim 26, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:64 (FIG. 51); SEQ ID NO:65 (FIG. 52); SEQ ID NO:66 (FIG. 53); SEQ ID NO:67 (FIG. 54); SEQ ID NO:68 (FIG. 55); SEQ ID NO:75 (FIG. 63); and SEQ ID NO:73 (FIG. 61).

28. The expression cassette of claim 26, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:48 (FIG. 35); SEQ ID NO:49 (FIG. 36); SEQ ID NO:50 (FIG. 37); SEQ ID NO:76 (FIG. 64); and SEQ ID NO:74 (FIG. 62).

29. The expression cassette of claim 14, wherein said Env polypeptide includes a gp140 Env polypeptide or a polypeptide derived from a gp140 Env polypeptide.

30. The expression cassette of claim 29, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:56 (FIG. 43); SEQ ID NO:57 (FIG. 44); SEQ ID NO:58 (FIG. 45); SEQ ID NO:59 (FIG. 46); SEQ ID NO:60 (FIG. 47); SEQ ID NO:61 (FIG. 48); SEQ ID NO:62 (FIG. 49); and SEQ ID NO:63 (FIG. 50).

31. The expression cassette of claim 29, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:36 (FIG. 23); SEQ ID NO:37 (FIG. 24); SEQ ID NO:38 (FIG. 25); SEQ ID NO:39 (FIG. 26); SEQ ID NO:40 (FIG. 27); SEQ ID NO:41 (FIG. 28); SEQ ID NO:42 (FIG. 29); SEQ ID NO:43 (FIG. 30); SEQ ID NO:44 (FIG. 31); SEQ ID NO:45 (FIG. 32); SEQ ID NO:46 (FIG. 33); and SEQ ID NO:47 (FIG. 34).

32. The expression cassette of claim 14, wherein said Env polypeptide includes a gp120 Env polypeptide or a polypeptide derived from a gp120 Env polypeptide.

33. The expression cassette of claim 32, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:54 (FIG. 41); and SEQ ID NO:55 (FIG. 42).

34. The expression cassette of claim 32, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting

(FIG. 21).

35. The expression cassette of claim 14, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:55 (FIG. 42); SEQ ID NO:62 (FIG. 49); SEQ ID NO:63 (FIG. 50); and SEQ ID NO:68 (FIG. 55).

36. A recombinant expression system for use in a selected host cell, comprising, an expression cassette of claim 1, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected host cell.

37. The recombinant expression system of claim 36, wherein said control elements are selected from the group consisting of a transcription promoter, a transcription enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences.

38. The recombinant expression system of claim 36, wherein said transcription promoter is selected from the group consisting of CMV, CMV+intron A, SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein.

39. A cell comprising an expression cassette of claim 1, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected cell.

40. The cell of claim 39, wherein the cell is a mammalian cell.

41. The cell of claim 40, wherein the cell is selected from the group consisting of BHK, VERO, HT1080, 293, RD, COS-7, and CHO cells.

42. The cell of claim 41, wherein said cell is a CHO cell.

43. The cell of claim 39, wherein the cell is an insect cell.

44. The cell of claim 43, wherein the cell is either Trichoplusia ni (Tn5) or Sf9 insect cells.

45. The cell of claim 39, wherein the cell is a bacterial cell.

46. The cell of claim 39, wherein the cell is a yeast cell.

47. The cell of claim 39, wherein the cell is a plant cell.

48. The cell of claim 39, wherein the cell is an antigen presenting cell.

49. The cell of claim 48, wherein the lymphoid cell is selected from the group consisting of macrophage, monocytes, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof.

50. The cell of claim 39, wherein the cell is a primary cell.

51. The cell of claim 39, wherein the cell is an immortalized cell.

52. The cell of claim 39, wherein the cell is a tumor-derived cell.

53. A method for producing a polypeptide including HIV Gag polypeptide sequences, said method comprising, incubating the cells of claim 39, under conditions for producing said polypeptide.

54. A method for producing **virus-like particles (VLPs)**, comprising, incubating the cells of claim 39, under conditions for producing said **VLPs**.

55. A method for producing a composition of **virus-like particles (VLPs)**, comprising, (a) incubating the cells of claim 39, under conditions for producing said **VLPS**; and (b) substantially purifying said **VLPs** to produce a composition of **VLPs**.

56. A cell line useful for packaging lentivirus vectors, comprising suitable host cells that have been transfected with an expression vector containing an expression cassette of claim 1, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell.

57. A cell line useful for packaging lentivirus vectors, comprising suitable host cells that have been transfected with an expression vector containing an expression cassette of claim 2, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell.

58. A cell line useful for packaging lentivirus vectors, comprising suitable host cells that have been transfected with an expression vector containing an expression cassette of claim 3, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell.

59. A cell line useful for packaging lentivirus vectors, comprising suitable host cells that have been transfected with an expression vector containing an expression cassette of claim 11, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell.

60. A gene delivery vector for use in a Mammalian subject, comprising a suitable gene delivery vector for use in said subject, wherein the vector comprises an expression cassette of claim 1, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the subject.

61. A method of DNA immunization of a subject, comprising, introducing a gene delivery vector of claim 60 into said subject under conditions that are compatible with expression of said expression cassette in said subject.

62. The method of claim 61, wherein said gene delivery vector is a nonviral vector.

63. The method of claim 61, wherein said vector is delivered using a particulate carrier.

64. The method of claim 63, wherein said vector is coated on a gold or tungsten particle and said coated particle is delivered to said subject using a gene gun.

65. The method of claim 63, wherein said vector is encapsulated in a liposome preparation.

66. The method of claim 61, wherein said vector is a viral vector.

67. The method of claim 66, wherein said viral vector is a retroviral vector.

68. The method of claim 67, wherein said viral vector is a lentiviral vector.

69. The method of claim 61, wherein said subject is a mammal.

70. The method of claim 69, wherein said mammal is a human.

71. A method of generating an immune response in a subject, comprising transfecting cells of said subject a gene delivery vector of claim 60, under conditions that permit the expression of said polynucleotide and production of said polypeptide, thereby eliciting an immunological response to said polypeptide.

72. The method of claim 71, wherein said vector is a nonviral vector.

73. The method of claim 72, wherein said vector is delivered using a particulate carrier.

74. The method of claim 73, wherein said vector is coated on a gold or tungsten particle and said coated particle is delivered to said vertebrate cell using a gene gun.

75. The method of claim 73, wherein said vector is encapsulated in a liposome preparation.

76. The method of claim 71, wherein said vector is a viral vector.

77. The method of claim 76, wherein said viral vector is a retroviral vector.

78. The method of claim 77, wherein said viral vector is a lentiviral vector.

79. The method of claim 71, wherein said subject is a mammal.

80. The method of claim 79, wherein said mammal is a human.

81. The method of claim 71, wherein said transfecting is done ex vivo and said transfected cells are reintroduced into said subject.

82. The method of claim 71, wherein said transfecting is done in vivo in said subject.

83. The method of claim 71, where said immune response is a humoral immune response.

84. The method of claim 71, where said immune response is a cellular immune response.

85. A gene delivery vector comprising an alphavirus vector construct, wherein said alphavirus construct comprises an expression cassette according to claim 1.

86. The gene delivery vector of claim 85, wherein the alphavirus vector construct is a cDNA vector construct.

87. The gene delivery vector of claim 85, wherein the alphavirus comprises a recombinant alphavirus particle preparation.

88. The gene delivery vector of claim 85, wherein the vector comprises a eukaryotic layered vector initiation system.

89. A method of stimulating an immune response in a subject comprising administering the gene delivery vector of claim 85 in an amount effective to stimulate an immune response in said subject.

90. The method of claim 89, wherein the gene delivery vector is administered intramuscularly, intramucosally, intranasally, subcutaneously, intradermally, transdermall, intravaginally, intrarectally, orally or intravenously.

L22 ANSWER 13 OF 17 USPATFULL on STN
2003:78090 Molecular antigen array.
Sebbel, Peter, Zurich, SWITZERLAND
Dunant, Nicolas, Zurich, SWITZERLAND
Bachmann, Martin, Winterthur, SWITZERLAND
Tissot, Alain, Zurich, SWITZERLAND
Lechner, Franziska, Zurich, SWITZERLAND
Renner, Wolfgang A., Zurich, SWITZERLAND
Hennecke, Frank, Zurich, SWITZERLAND
Nieba, Lars, Herisau, SWITZERLAND
Cytos Biotechnology AG (non-U.S. corporation)
US 2003054010 A1 20030320
APPLICATION: US 2001-848616 A1 20010504 (9)
PRIORITY: US 2000-202341P 20000505 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A composition comprising a bacterial pilus to which an antigen or antigenic determinant has been attached by a covalent bond.

2. The composition of claim 1, wherein said covalent bond is not a peptide bond.

3. The composition of claim 1, wherein said bacterial pilus is a Type-1 pilus of Escherichia coli.

4. The composition of claim 1, wherein pilin subunits of said Type-1 pilus comprises the amino acid sequence shown in SEQ ID NO:146 or a sequence having at least 65, 70, 75, 80, 85, 90 or 95% sequence identity to SEQ ID NO:146.

5. The composition of claim 1, wherein said bacterial pilus and said antigen or antigen determinant are attached via a non-naturally occurring attachment.

6. The composition of claim 1, wherein said attachment comprises an organizer comprising at least one first attachment site, and wherein said organizer is connected to said pilus by at least one covalent bond.

7. The composition of claim 6, wherein said organizer is a polypeptide or a residue thereof, and wherein said second attachment site is a polypeptide or a residue thereof.

8. The composition of claim 6, wherein said first and/or a second attachment sites comprise: (a) an antigen and an antibody or antibody fragment thereto; (b) biotin and avidin; (c) strepavidin and biotin, (d) a receptor and its ligand; (e) a ligand-binding protein and its ligand; (f) interacting leucine zipper polypeptides; (g) an amino group and a chemical group reactive thereto; (h) a carboxyl group and a chemical group reactive thereto; (i) a sulfhydryl group and a chemical group reactive thereto; or (j) a combination thereof.

9. The composition of claim 1, wherein said bacterial pilus and said

antigen or antigenic determinant are attached by an attachment comprising interacting leucine zipper polypeptides.

10. The composition of claim 5, wherein interacting leucine zipper polypeptides are JUN and/or FOS leucine zipper polypeptides.

11. A composition comprising a bacterial pilin polypeptide to which an antigen or antigenic determinant has been attached by a covalent bond.

12. The composition of claim 11, wherein said covalent bond is not a peptide bond.

13. The composition of claim 11, wherein said polypeptide is from a Type-1 pilus of Escherichia coli.

14. The composition of claim 11, wherein said bacterial pilin polypeptide comprises the amino acid sequence shown in SEQ ID NO:146 or a sequence having at least 65, 70, 75, 80, 85, 90 or 95% sequence identity to SEQ ID NO:146.

15. The composition of claim 11, wherein said bacterial pilin polypeptide and said antigen or antigenic determinant are attached by a non-naturally occurring attachment.

16. The composition of claim 11, wherein said attachment comprises an organizer comprising at least one first attachment site, and wherein said organizer is connected to said pilus by at least one covalent bond.

17. The composition of claim 16, wherein said organizer is a polypeptide or a residue thereof, and wherein said second attachment site is a polypeptide or a residue thereof.

18. The composition of claim 11, wherein said first and/or a second attachment sites comprise: (a) an antigen and an antibody or antibody fragment thereto; (b) biotin and avidin; (c) strepavidin and biotin; (d) a receptor and its ligand; (e) a ligand-binding protein and its ligand; (f) interacting leucine zipper polypeptides; (g) an amino group and a chemical group reactive thereto; (h) a carboxyl group and a chemical group reactive thereto; (i) a sulfhydryl group and a chemical group reactive thereto; or (j) a combination thereof.

19. The composition of claim 15, wherein said attachment comprises interacting leucine zipper polypeptides.

20. The composition of claim 13, wherein said interacting leucine zipper polypeptides are JUN and/or FOS leucine zipper polypeptides.

21. A composition comprising: (a) a non-natural molecular scaffold comprising: (i) a core particle selected from the group consisting of: (1) a bacterial pilus or pilin protein; and (2) a recombinant form of a bacterial pilus or pilin protein; and (ii) an organizer comprising at least one first attachment site, wherein said organizer is connected to said core particle by at least one covalent bond; and (b) an antigen or antigenic determinant with at least one second attachment site, said second attachment site being selected from the group consisting of: (i) an attachment site not naturally occurring with said antigen or antigenic determinant; and (ii) an attachment site naturally occurring with said antigen or antigenic determinant, wherein said second attachment site is capable of association through at least one non-peptide bond to said first attachment site; and wherein said antigen or antigenic determinant and said scaffold interact through said association to form an ordered and repetitive antigen array.

22. The composition of claim 21, wherein said organizer is a polypeptide or residue thereof; and wherein said second attachment site is a polypeptide or residue thereof.

23. The composition of claim 21, wherein said first and/or said second attachment sites comprise: (a) an antigen and an antibody or antibody fragment thereto; (b) biotin and avidin; (c) strepavidin and biotin; (d) a receptor and its ligand; (e) a ligand-binding protein and its ligand; (f) interacting leucine zipper polypeptides; (g) an amino group and a chemical group reactive thereto; (h) a carboxyl group and a chemical group reactive thereto; (i) a sulfhydryl group and a chemical group reactive thereto; or (j) a combination thereof.

24. The composition of claim 21, wherein said first and/or said second attachment sites comprise interacting leucine zipper polypeptides.

25. The composition of claim 21, wherein said bacterial pilus is a Type-1 pilus of Eschericia coli.

26. The composition of claim 21, wherein pilus subunits of said type-1

plus comprises the amino acid sequence of SEQ ID NO: 146 or a sequence having at least 65, 70, 75, 80, 85, 90 or 95% sequence identity to SEQ ID NO:146.

27. The composition of claim 26, wherein said interacting leucine zipper polypeptides are the JUN and/or FOS leucine zipper polypeptides.

28. A composition comprising: (a) a non-natural molecular scaffold comprising: (i) a **virus-like particle** that is a dimer or a multimer of a polypeptide comprising amino acids 1-147 of SEQ ID NO:158 as core particle or a sequence having at least 65, 70, 75, 80, 85, 90 or 95% sequence identity to SEQ ID NO:158; and (ii) an organizer comprising at least one first attachment site, wherein said organizer is connected to said core particle by at least one covalent bond; and (b) an antigen or antigenic determinant with at least one second attachment site, said second attachment site being selected from the group consisting of: (i) an attachment site not naturally occurring with said antigen or antigenic determinant; and (ii) an attachment site naturally occurring with said antigen or antigenic determinant, wherein said second attachment site is capable of association through at least one non-peptide bond to said first attachment site; and wherein said antigen or antigenic determinant and said scaffold interact through said association to form an ordered and repetitive antigen array.

29. The composition of claim 28, wherein said organizer is a polypeptide or residue thereof; and wherein said second attachment site is a polypeptide or residue thereof.

30. The composition of claim 28, wherein said first and/or said second attachment sites comprise: (a) an antigen and an antibody or antibody fragment thereto; (b) biotin and avidin; (c) strepavidin and biotin; (d) a receptor and its ligand; (e) a ligand-binding protein and its ligand; (f) interacting leucine zipper polypeptides; (g) an amino group and a chemical group reactive thereto; (h) a carboxyl group and a chemical group reactive thereto; (i) a sulfhydryl group and a chemical group reactive thereto; or a combination thereof.

31. The composition of claim 30, wherein said first attachment site is an amino group and said second attachment site is a sulfhydryl group.

32. The composition of claim 30, wherein said **virus-like particle** and said antigen or antigenic determinant are attached by an attachment comprising interacting leucine zipper polypeptides.

33. The composition of claim 32, wherein said interacting leucine zipper polypeptides are JUN and/or FOS FOS polypeptides.

34. A composition comprising: (a) a non-natural molecular scaffold comprising: (i) Hepatitis B virus capsid protein comprising an amino acid sequence selected from the group consisting of: (1) the amino acid sequence of SEQ ID NO:89; (2) the amino acid sequence of SEQ ID NO:90; (3) the amino acid sequence of SEQ ID NO:93; (4) the amino acid sequence of SEQ ID NO:98; (5) the amino acid sequence of SEQ ID NO:99; (6) the amino acid sequence of SEQ ID NO: 102; (7) the amino acid sequence of SEQ ID NO: 104; (8) the amino acid sequence of SEQ ID NO:105; (9) the amino acid sequence of SEQ ID NO:106, (10) the amino acid sequence of SEQ ID NO:119; (11) the amino acid sequence of SEQ ID NO:120; (12) the amino acid sequence of SEQ ID NO:123; (13) the amino acid sequence of SEQ ID NO:125, (14) the amino acid sequence of SEQ ID NO:131; (15) the amino acid sequence of SEQ ID NO:132, (16) the amino acid sequence of SEQ ID NO:134, (17) the amino acid sequence of SEQ ID NO:157; and (18) the amino acid sequence of SEQ ID NO:158; and (ii) an organizer comprising at least one first attachment site, wherein said organizer is connected to said core particle by at least one covalent bond; and (b) an antigen or antigenic determinant with at least one second attachment site, said second attachment site being selected from the group consisting of: (i) an attachment site not naturally occurring with said antigen or antigenic determinant; and (ii) an attachment site naturally occurring with said antigen or antigenic determinant, wherein said second attachment site is capable of association through at least one non-peptide bond to said first attachment site; and wherein said antigen or antigenic determinant and said scaffold interact through said association to form an ordered and repetitive antigen array.

35. The composition of claim 34, wherein said organizer is a polypeptide or residue thereof, wherein said second attachment site is a polypeptide or residue thereof, and wherein said first attachment site is a lysine residue and said second attachment site is a cysteine residue.

36. The composition of claim 34, wherein one or more cysteine residues of said Hepatitis B virus capsid protein have been either deleted or substituted with another amino acid residue.

37. The composition of claim 34, wherein said first and/or said second attachment sites comprise: (a) an antigen and an antibody or antibody fragment thereto; (b) biotin and avidin; (c) strepavidin and biotin; (d) a receptor and its ligand; (e) a ligand-binding protein and its ligand; (f) interacting leucine zipper polypeptides; (g) an amino group and a chemical group reactive thereto; (h) a carboxyl group and a chemical group reactive thereto; (i) a sulfhydryl group and a chemical group reactive thereto, or (j) a combination thereof.

38. The composition of claim 36, wherein the cysteine residues corresponding to amino acids 48 and 107 in SEQ ID NO:134 have been either deleted or substituted with another amino acid residue.

39. The composition of claim 37, wherein said Hepatitis B virus capsid protein and said antigen or antigenic determinant are attached by an attachment comprising interacting leucine zipper polypeptides.

40. The composition of claim 39, wherein said interacting leucine zipper polypeptides are FOS and/or JUN polypeptides.

41. The composition of any one of claims 28, 34, 35, 36 and 38, wherein said antigen is selected from the group consisting of: (a) an antigen suited to induce an immune response against bacteria, (b) an antigen suited to induce an immune response against viruses, (c) an antigen suited to induce an immune response against parasites, (d) an antigen suited to induce an immune response against cancer cells, (e) an antigen suited to induce an immune response against allergens, (f) an antigen suited to induce an immune response in a farm animals, and (g) a protein suited to induce an immune response in a pet.

42. The composition of claim 41, wherein the antigen is a protein, polypeptide, or a fragment thereof.

43. The composition of claim 41, wherein said antigen induces an immune response against one or more allergens.

44. The composition of claim 41, wherein said antigen is: (a) a recombinant protein of HIV, (b) a recombinant protein of Influenza virus, (c) a recombinant protein of Hepatitis C virus, (d) a recombinant protein of Toxoplasma, (e) a recombinant protein of Plasmodium falciparum, (f) a recombinant protein of Plasmodium vivax, (g) a recombinant protein of Plasmodium ovale, (h) a recombinant protein of Plasmodium malariae, (i) a recombinant protein of breast cancer cells, (j) a recombinant protein of kidney cancer cells, (k) a recombinant protein of prostate cancer cells, (l) a recombinant protein of skin cancer cells, (m) a recombinant protein of brain cancer cells, (n) a recombinant protein of leukemia cells, (o) a recombinant profiling, (p) a recombinant protein of bee sting allergy, (q) a recombinant protein of nut allergy, (r) a recombinant protein of food allergies, (s) a recombinant protein of asthma, or (t) a recombinant protein of Chlamydia.

45. The composition of any one of claims 1, 11 and 21, wherein said antigen is selected from the group consisting of: (a) an antigen suited to induce an immune response against bacteria, (b) an antigen suited to induce an immune response against viruses, (c) an antigen suited to induce an immune response against parasites, (d) an antigen suited to induce an immune response against cancer cells, (e) an antigen suited to induce an immune response in a farm animals, and (f) an antigen suited to induce an immune response in a pet, and (g) any other antigen involved in a pathophysiological context.

46. The composition of claim 45, wherein the antigen is a protein, a polypeptide, or a fragment thereof.

47. The composition of any one of claims 1, 11 or 21, wherein said antigen is: (a) a recombinant protein of HIV, (b) a recombinant protein of Influenza virus, (c) a recombinant protein of Hepatitis C virus, (d) a recombinant protein of Toxoplasma, (e) a recombinant protein of Plasmodium falciparum, (f) a recombinant protein of Plasmodium vivax, (g) a recombinant protein of Plasmodium ovale, (h) a recombinant protein of Plasmodium malariae, (i) a recombinant protein of breast cancer cells, (j) a recombinant protein of kidney cancer cells, (k) a recombinant protein of prostate cancer cells, (l) a recombinant protein of skin cancer cells, (m) a recombinant protein of brain cancer cells, (n) a recombinant protein of leukemia cells, (o) a recombinant profiling, (p) a recombinant protein of Chlamydia.

48. A pharmaceutical composition comprising the composition of any one of claims 1, 11, 21, 28, 34, 35, 36, 38, 41 or 44, and a pharmaceutically acceptable carrier.

49. A vaccine composition comprising the composition of any one of claims 1, 11, 21, 28, 34, 35, 36, 38, 41 or 44.

50. The vaccine composition of claim 49, further comprising at least one adjuvant.

51. A method of immunizing, comprising administering to a subject the vaccine composition of claim 49 or 50.

52. The method of claim 51, wherein said administering produces an immune response.

53. The method of claim 51, wherein said administering produces a humoral immune response.

54. The method of claim 51, wherein said administering produces a cellular immune response.

55. The method of claim 51, wherein said administering produces a humoral immune response and a cellular immune response.

56. The method of claim 51, wherein said administering produces a protective immune response.

57. A method of making the composition of claim 1, comprising combining said pilus and said antigen or antigenic determinant, wherein said pilus and said antigen or antigenic determinant interact to form an antigen array.

58. The method of claim 57, wherein said antigen array is ordered and/or repetitive.

59. A method of making the composition of claim 11, comprising combining said pilin polypeptide and said antigen or antigenic determinant, wherein said pilin polypeptide and said antigen or antigenic determinant interact to form an antigen array.

60. The method of claim 59, wherein said antigen array is ordered and/or repetitive.

61. A method of making the composition of claim 21, 28, 34, 35, 36 or 38, comprising combining said non-natural molecular scaffold and said antigen or antigenic determinant, wherein said non-natural molecular scaffold and said antigen or antigenic determinant interact to form an antigen array.

62. The method of claim 61, wherein said antigen array is ordered and/or repetitive.

63. A composition comprising: (a) a non-natural molecular scaffold comprising: (i) a core particle selected from the group consisting of: (1) a bacterial pilus; and (2) a recombinant form of a bacterial pilus or pilin protein; and (ii) an organizer comprising at least one first attachment site, wherein said organizer is connected to said core particle by at least one covalent bond; and (b) an antigen or antigenic determinant with at least one second attachment site, said second attachment site being selected from the group consisting of: (i) an attachment site not naturally occurring with said antigen or antigenic determinant; and (ii) an attachment site naturally occurring with said antigen or antigenic determinant, wherein said second attachment site is capable of association through at least one non-peptide bond to said first attachment site; wherein said antigen or antigenic determinant and said scaffold interact through said association to form an ordered and repetitive antigen array, and wherein said antigen or antigenic determinant is selected from the group consisting of an influenza M2 peptide, the GRA2 polypeptide, the DP178c peptide, the tumor necrosis factor polypeptide, a tumor necrosis factor peptide, the B2 peptide, the D2 peptide, and the Aβ peptide.

64. The composition of claim 63, wherein said antigen or antigenic determinant is the influenza M2 peptide or variants thereof.

65. The composition of claim 63, wherein said antigen or antigenic determinant is the GRA2 polypeptide.

66. The composition of claim 63, wherein said antigen or antigenic determinant is the DP178c peptide.

67. The composition of claim 63, wherein said antigen or antigenic determinant is the tumor necrosis factor polypeptide.

68. The composition of claim 63, wherein said antigen or antigenic determinant is a tumor necrosis factor peptide.

69. The composition of claim 63, wherein said antigen or antigenic determinant is the B2 peptide.

70. The composition of claim 63, wherein said antigen or antigenic determinant is the D2 peptide.

71. The composition of claim 63, wherein said antigen or antigenic determinant is the Aβ peptide.

72. The composition of claim 63, wherein said organizer is a polypeptide or residue thereof; and wherein said second attachment site is a polypeptide or residue thereof.

73. The composition of claim 63, wherein said first and/or said second attachment sites comprise: (a) an antigen and an antibody or antibody fragment thereto; (b) biotin and avidin; (c) strepavidin and biotin; (d) a receptor and its ligand, (e) a ligand-binding protein and its ligand; (f) interacting leucine zipper polypeptides; (g) an amino group and a chemical group reactive thereto; (h) a carboxyl group and a chemical group reactive thereto; (i) a sulfhydryl group and a chemical group reactive thereto; or (j) a combination thereof.

74. The composition of claim 63, wherein said first and/or said second attachment sites comprise interacting leucine zipper polypeptides.

75. The composition of claim 63, wherein said bacterial pilus is a Type-1 pilus of Eschericia coli.

76. The composition of claim 63, wherein pilus subunits of said type-1 pilus comprise the amino acid sequence of SEQ ID No 146 or a sequence having at least 65, 70, 75, 80, 85, 90 or 95% sequence identity to SEQ ID NO:146.

77. The composition of claim 63, wherein said interacting leucine zipper polypeptides are the JUN and/or FOS leucine zipper polypeptides.

78. A vaccine composition comprising the composition of claim 63 or claim 43.

79. A method of immunizing, comprising administering to a subject the vaccine composition of claim 49 or 50.

80. The method of claim 79, wherein said administering produces an immune response.

81. A method of making the composition of claim 63, comprising combining said non-natural molecular scaffold and said antigen or antigenic determinant, wherein said non-natural molecular scaffold and said antigen or antigenic determinant interact to form an antigen array.

82. The method of claim 81, wherein said antigen array is ordered and/or repetitive.

83. A method of immunizing, comprising administering the composition of any one of claims 1, 11, 21, 49 or 50 to a subject, wherein for inducing a Th2 response, wherein said administering produces a Th2 response that is specific for said antigen or antigenic determinant.

84. The method of claim 83, wherein antibodies specific for said antigen or antigenic determinant of a subtype corresponding to the Th2 subtype are induced in the subject.

85. The method of claim 83, wherein the subject does not generate a Th1 response that is specific for said pilus, said pilin polypeptide, or said antigen or antigenic determinant.

L22 ANSWER 14 OF 17 USPATFULL on STN
2002:112288 Isolated amphiphilic peptides derived from the cytoplasmic tail of viral envelope proteins.
Rozenberg, Yanina, Studio City, CA, UNITED STATES
Anderson, W. French, San Marino, CA, UNITED STATES
US 2002058020 A1 20020516
APPLICATION: US 2001-756250 A1 20010108 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An isolated peptide comprising a fragment of a viral envelope protein, wherein said peptide is free of the portion of the envelope protein N-terminal of the membrane-spanning region of the envelope protein, said peptide having a membrane-destabilizing activity.

2. The peptide of claim 1 wherein the membrane destabilizing activity of said peptide is sufficient to induce an electrophysiologically detectable increase of the release of a suitable marker from a liposome at an active concentration of 30 mM peptide/1 mol lipid in a suitable assay.

3. The peptide of claim 2, wherein said peptide forms an α-helical amphiphilic structure.

4. The peptide of claim 3 having a hydrophobic moment μ of at least 0.9 as calculated using DNASIS software employing the Chou, Fasman and Rose algorithm and calculated with the Kyte and Doolittle algorithm.

5. The peptide of claim 4 wherein said fragment comprises at least 8 amino acids.

6. The peptide of claim 5 wherein said fragment comprises at least the first 8 amino acids of the N-terminal portion of the cytoplasmic tail region of the envelope protein.

7. The peptide of claim 6 wherein said fragment comprises at least one out-of-phase residue.

8. The peptide of claims 7 wherein a portion of said peptide is present in said membrane-spanning region of said viral envelope protein.

9. The peptide of claim 8 wherein said peptide comprises the amino acid sequence of SEQ ID NO: 1.

10. Derivatives and analogues of the peptide of claim 1 having at least one substitution of an amino acid residue that maintains the membrane-destabilizing activity of said peptide and/or having the reverse sequence of said peptide.

11. Use of the peptide of claim 1, or a nucleic acid encoding said peptide, for the preparation of a viral or synthetic vector.

12. Use of the peptide of claim 1, or a nucleic acid encoding said peptide, for the preparation of a medicament.

13. Use of an amphiphilic compound having a membrane-destabilizing activity for the preparation of a viral vector.

14. A peptide selected from the group consisting of (SEQ ID NO: 2) and (SEQ ID NO: 3) and derivatives and analogues of (SEQ ID NO: 2) and (SEQ ID NO: 3) having at least one amino acid substitution of (SEQ ID NO: 2) and (SEQ ID NO: 3) that maintains the membrane-destabilizing activity of said peptide.

15. The peptide of claim 14 wherein said peptide is (SEQ ID NO: 2).

16. The peptide of claim 14 wherein said peptide is (SEQ ID NO: 3).

17. A viral particle including a modified envelope protein, wherein said modified envelope protein includes the peptide of claim 1, wherein said peptide is located in a portion of said envelope protein external to the viral membrane.

18. The viral particle of claim 17 wherein said modified envelope protein further includes a targeting polypeptide including a binding region that binds to a liqand.

19. A **retroviral vector particle** including a retroviral envelope protein and the peptide of claim 1, wherein said peptide is attached to the retroviral membrane.

20. The **retroviral vector particle** of claim 19 wherein said retroviral envelope protein is a modified envelope protein that includes a targeting polypeptide that binds to a ligand.

21. A **retroviral vector particle** including a retroviral envelope protein, a targeting polypeptide including a binding region that binds to a ligand, and the peptide of claim 1, wherein each of said targeting polypeptide and the peptide of claim 1 is attached to the retroviral membrane.

22. A **retroviral vector particle** including a retroviral envelope protein, and a polypeptide including a targeting polypeptide including a binding region that binds to a ligand, a spacer moiety, and the peptide of claim 1, wherein said polypeptide is attached to the retroviral membrane.

23. A **retroviral vector particle** including (i) a targeting

polypeptide including a binding region that binds to a ligand and (ii) the peptide of claim 1, wherein each of said targeting polypeptide and the peptide of claim 1 is attached separately to the membrane of said **retroviral vector particle**, and said **retroviral vector particle** does not include a retroviral envelope protein.

24. A **retroviral vector particle** including a polypeptide including (i) a targeting polypeptide including a binding region that binds to a ligand, (ii) a spacer moiety, and (iii) the peptide of claim 1, wherein said polypeptide is attached to the membrane of said **retroviral vector particle**, and said **retroviral vector particle** does not include a retroviral envelope protein.

25. The viral particle of claim 17 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

26. The **retroviral vector particle** of claim 19 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

27. The **retroviral vector particle** of claim 21 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

28. The **retroviral vector particle** of claim 22 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

29. The **retroviral vector particle** of claim 23 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

30. The **retroviral vector particle** of claim 24 wherein said particle further includes at least one polynucleotide encoding a therapeutic agent.

31. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the viral particle of claim 25.

32. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the **retroviral vector particle** of claim 26.

33. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the **retroviral vector particle** of claim 27.

34. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the **retroviral vector particle** of claim 28.

35. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the **retroviral vector particle** of claim 29.

36. A method of expressing a therapeutic agent in an animal, comprising: administering to an animal the **retroviral vector particle** of claim 30.

37. A packaging cell including a polynucleotide encoding the retroviral gag protein, a polynucleotide encoding the retroviral pol protein, and a polynucleotide encoding a viral envelope protein including the peptide of claim 1.

38. The cell of claim 37 wherein said viral envelope protein further includes a targeting polypeptide including a binding region that binds to a ligand.

39. A producer cell formed from the packaging cell of claim 37.

40. A producer cell formed from the packaging cell of claim 38.

41. A packaging cell including a polynucleotide encoding the retroviral gag protein, a polynucleotide encoding the retroviral pol protein, a polynucleotide encoding the retroviral env protein, a polynucleotide including a nucleic acid sequence encoding the peptide of claim 1 and a nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein, and a polynucleotide including a nucleic acid sequence encoding a targeting polypeptide including a binding region which binds to a ligand and a nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein.

42. A producer cell formed from the packaging cell of claim 41.

43. A packaging cell including a polynucleotide encoding the retroviral gag protein, a polynucleotide encoding the retroviral pol protein, a polynucleotide encoding the retroviral env protein, and a polynucleotide including a first nucleic acid sequence encoding the peptide of claim 1,

a second nucleic acid sequence encoding a spacer moiety, a third nucleic acid sequence encoding a targeting polypeptide including a binding region that binds to a ligand, and a fourth nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein.

44. A producer cell formed from the packaging cell of claim 43.

45. A pre-packaging cell including a polynucleotide encoding the retroviral gag protein, a polynucleotide encoding the retroviral pol protein, a polynucleotide including a nucleic acid sequence encoding the peptide of claim 1 and a nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein, and a polynucleotide including a nucleic acid sequence encoding a targeting polypeptide including a binding region that binds to a ligand and a nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein.

46. A pre-packaging cell line including a polynucleotide encoding the retroviral gag protein, a polynucleotide encoding the retroviral pol protein, and a polynucleotide including (i) a first nucleic acid sequence encoding the peptide of claim 1, (ii) a second nucleic acid sequence encoding a spacer moiety, (iii) a third nucleic acid sequence encoding a targeting polypeptide including a binding region that binds to a ligand, and (iv) a fourth nucleic acid sequence encoding a membrane-spanning region of a viral envelope protein.

L22 ANSWER 15 OF 17 USPATFULL on STN
2001:229423 Highly efficient gene transfer into human repopulating stem cells by RD114 pseudotyped **retroviral vector particles**.
Kelly, Patrick F., Cordova, TN, United States
Vanin, Elio F., Memphis, TN, United States
St. Jude Children's Research Hospital (U.S. corporation)
US 2001051375 A1 20011213
APPLICATION: US 2001-801302 A1 20010307 (9)
PRIORITY: US 2000-187534P 20000307 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A highly efficient method for transducing stem cells with a vector particle containing a gene of interest, which method comprises contacting target stem cells with vector particles pseudotyped with feline endogenous virus RD114 envelope protein and containing a gene of interest, wherein the vector particles are substantially free of factors that induce stem cell differentiation.

2. The method of claim 1, wherein the vector particle is a **retroviral vector particle** comprising a modified retroviral genome containing the gene of interest.

3. The method of claim 2, wherein the **retroviral vector particles** are freed of factors that induce stem cell differentiation by being substantially free of producer cells and producer cell supernatant.

4. The method of claim 3, wherein the retroviral particles are pre-adsorbed onto a surface that promotes adherence of the retroviral particles.

5. The method of claim 4, wherein the surface is coated with an adherence promoting agent.

6. The method of claim 5, wherein the adherence promoting agent is retronectin.

7. The method of claim 2, wherein the retroviral particles are freed of producer cells and producer cell supernatant by ultracentrifugation.

8. The method of claim 2 wherein the retroviral particle is an oncoviral particle.

9. The method of claim 2 wherein the retroviral particle is a lentiviral particle.

10. The method of claim 1 wherein the target stem cells are pre-stimulated.

11. The method of claim 10, wherein the target stem cells are prestimulated by treatment with signaling molecules selected from the group consisting of cytokines, growth factors and phytohemagglutinin.

12. The method of claim 1 wherein the target stem cells are hematopoietic stem cells.

13. The method of claim 12 wherein the target hematopoietic stem cells are selected from the group consisting of cord blood cells, mobilized peripheral blood cells, bone marrow cells, and liver.

14. The method of claim 13, wherein the target hematopoietic stem cells are selected from the group consisting of CD34+ cells and CD34+ CD38- cells.

15. The method according to claim 2, wherein upon engraftment of the transduced stem cells contacted one time with the retroviral particles into a host, greater than 10% of the transduced cells express the gene of interest.

16. The method according to claim 15, wherein greater than about 40% of the transduced cells express the gene of interest.

17. A population of stem cells transduced with vector particles pseudotyped with feline endogenous virus RD114 envelope protein and containing a gene of interest, wherein the population of stem cells are substantially undifferentiated.

18. The population of stem cells of claim 17, wherein the vector particle is a retroviral particle comprising a modified retroviral genome containing the gene of interest.

19. The population of stem cells of claim 18, wherein upon engraftment of the stem cells into a host, the number of stem cells in the host that express the gene of interest is greater than 10% times a number of exposures of the stem cells to the **retroviral vector particles**.

20. The population of stem cells of claim 18, wherein the stem cells were transduced by a single exposure to the **retroviral vector particles** and upon engraftment of the stem cells into a host, greater than about 40% of the stem cells express the gene of interest.

21. A method for introducing a gene of interest into a host, which method comprises introducing the transduced stem cells of claim 17 into a host.

22. The method according to claim 21, wherein the host is a human and the stem cells are human stem cells.

23. The method according to claim 21, wherein the host is an immunodeficient animal and the stem cells are human stem cells.

24. The method according to claim 21, wherein upon engraftment of the transduced stem cells contacted one time with the retroviral particles into a host, greater than 10% of the transduced cells express the gene of interest.

25. The method according to claim 24, wherein greater than about 40% of the transduced stem cells express the gene of interest.

26. A method of treating a disease or disorder, which method comprises administering to a patient a therapeutically effective dose of the transduced stem cells of claim 17, wherein the gene of interest is a therapeutic gene.

27. The method of claim 26, wherein the disease or disorder is selected from the group consisting of hematopoietic disease, neural disease, joint-related disease, muscular disease, and liver disease.

28. A non-human animal engrafted with the stem cells of claim 17.

29. The non-human animal of claim 28, which is an immunodeficient mouse.

30. The non-human animal of claim 28, which is a monkey.

31. A kit comprising **retroviral vector particles** pseudotyped with feline endogenous virus RD114 envelope protein and containing a gene of interest their genome pre-adsorbed onto a surface that promotes adherence of the retroviral particles, wherein the **retroviral vector particles** are substantially free of producer cells and producer cell supernatant.

32. The kit of claim 31, wherein the surface is coated with an adherence promoting agent.

33. The kit of claim 32, wherein the adherence promoting agent is retronectin.

34. A method for preparing a kit comprising **retroviral vector particles** pseudotyped with feline endogenous virus RD114 envelope

protein and containing a gene of interest their genome pre-adsorbed onto a surface that promotes adherence of the retroviral particles, wherein the **retroviral vector particles** are substantially free of producer cells and producer cell supernatant, which method comprises contacting the surface with the **retroviral vector particles** for a sufficient period of time to permit adherence of the retroviral particles to the surface, and removing supernatant in which the retroviral particles were suspended from the surface.

35. The method of claim 34, wherein the surface is coated with an adherence promoting agent.

36. The method of claim 35, wherein the adherence promoting agent is retronectin.

37. The method of claim 34, further comprising storing the retroviral particles adsorbed onto the surface at -70° C.

L22 ANSWER 16 OF 17 USPATFULL on STN
1999:166839 Retroviral envelopes having modified hypervariable polyproline regions.
Anderson, W. French, San Marino, CA, United States
Wu, Bonnie Weimin, Pasadena, CA, United States
University of Southern California, Los Angeles, CA, United States (U.S. corporation)
US 6004798 19991221
APPLICATION: US 1997-856074 19970514 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A **retroviral vector particle** having a modified retroviral envelope polypeptide, said retroviral envelope polypeptide including the hypervariable polyproline region, wherein said hypervariable polyproline region is modified to include a targeting polypeptide including a binding region which binds to a ligand, and said **retroviral vector particle** including at least one polynucleotide encoding a therapeutic agent.

2. The **retroviral vector particle** of claim 1 wherein prior to modification the hypervariable polyproline region of said envelope has the sequence (SEQ ID NO:1), and in the modified polypeptide, amino acid residues 34 through 49 of (SEQ ID NO:1) are removed and replaced with the targeting polypeptide.

3. The **retroviral vector particle** of claim 1 wherein prior to modification the hypervariable polyproline region of said envelope has the sequence (SEQ ID NO:1), and in the modified polypeptide, amino acid residue 35 is changed from asparagine to glutamine, amino acid residue 48 is changed from threonine to glycine, and amino acid residue 49 is changed from serine to leucine, and the targeting polypeptide is inserted between amino acid residues 35 and 36 of (SEQ ID NO:1).

4. The **retroviral vector particle** of claim 1 wherein said ligand is an extracellular matrix component.

5. The **retroviral vector particle** of claim 4 wherein said extracellular matrix component is collagen.

6. The **retroviral vector particle** of claim 5 wherein said binding region which binds to collagen has the following structure: Trp-Arg-Glu-Pro-Ser-Phe-Met-Ala-Leu-Ser.

7. A modified polynucleotide encoding a modified retroviral envelope polypeptide, said retroviral envelope polypeptide including the hypervariable polyproline region, wherein, prior to modification, the polynucleotide encoding the hypervariable polyproline region encodes a hypervariable polyproline region having the sequence (SEQ ID NO:1), and in the modified polynucleotide, the codons encoding amino acid residues 34 through 49 of (SEQ ID NO:1) are removed and replaced with the polynucleotide encoding said targeting polypeptide.

8. A retroviral plasmid vector including the modified polynucleotide of claim 7.

9. A producer cell for producing a **retroviral vector particle** having a modified envelope polypeptide, said producer cell including the modified polynucleotide of claim 7.

10. A method of generating **retroviral vector particles,** comprising: (a) transfecting a cell line selected from the group consisting of (i) a pre-packaging cell line including polynucleotides encoding the gag and pol retroviral proteins; and (ii) a packaging cell

line including polynucleotides encoding the gag, pol, and env retroviral proteins with the retroviral plasmid vector of claim 8 to form a producer cell line; (b) culturing said producer cell line to generate **retroviral vector particles**; and (c) recovering said **retroviral vector particles** generated from said producer cell line.

11. A modified polynucleotide encoding a modified retroviral envelope polypeptide, said retroviral envelope polypeptide including the hypervariable polyproline region, wherein, prior to modification, the polynucleotide encoding the hypervariable polyproline region encodes a hypervariable polyproline region having the sequence (SEQ ID NO:1), and in the modified polynucleotide, the codon encoding amino acid residue 35 is changed such that the codon encoding amino acid residue 35 encodes glutamate, the codon encoding amino acid residue 48 is changed such that the codon encoding amino acid residue 48 encodes glycine, and the codon encoding amino acid residue 49 is changed such that the codon encoding amino acid residue 49 encodes leucine, and the polynucleotide encoding the targeting polypeptide is inserted between the codon encoding amino acid residue 35 and the codon encoding amino acid residue 36 of (SEQ ID NO: 1).

12. A retroviral plasmid vector including the modified polynucleotide of claim 11.

13. A producer cell for producing a **retroviral vector particle** having a modified envelope polypeptide, said producer cell including the modified polynucleotide of claim 11.

14. A method of generating **retroviral vector particles**, comprising: (a) transfecting a cell line selected from the group consisting of (i) a pre-packaging cell line including polynucleotides encoding the gag and pol retroviral proteins; and (ii) a packaging cell line including polynucleotides encoding the gag, pol, and env retroviral proteins with the retroviral plasmid vector of claim 12 to form a producer cell line; (b) culturing said producer cell line to generate **retroviral vector particles**; and (c) recovering said **retroviral vector particles** generated from said producer cell line.

L22 ANSWER 17 OF 17 USPATFULL on STN
1999:110215 Retroviral vectors produced by producer cell lines resistant to lysis by human serum.
Pensiero, Michael, Dickerson, MD, United States
Collins, Mary K. L., London, United Kingdom
Cosset, Francois-Loic, London, United Kingdom
Takeuchi, Yasuhiro, London, United Kingdom
Weiss, Robin A., London, United Kingdom
Genetic Therapy, Inc., Gaithersburg, MD, United States (U.S. corporation)Institute of Cancer Research Royal Cancer Hospital, London, United Kingdom (non-U.S. corporation)
US 5952225 19990914
APPLICATION: US 1995-516163 19950817 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A packaging cell for producing retroviral vectors resistant to inactivation by human serum, said packaging cell comprising a cell resistant to lysis by human serum, said packaging cell containing a polynucleotide encoding a portion of a virus selected from the group consisting of the feline endogenous virus RD114, BaEV, SSAV, FeLY-B, NZB virus, avian leukosis virus, and HVJ virus, said portion of said virus comprising at least the envelope protein, wherein said packaging cell does not include the entire viral RNA of the feline endogenous virus RD114, BeEV,SSAV, FeLV-B, NZB virus, avian leukosis virus, or HVJ virus.

2. The cell of claim 1 wherein said virus is the feline endogenous virus RD114.

3. The cell of claim 1 wherein said virus is BaEV.

4. The cell of claim 1 wherein said virus is SSAV.

5. The cell of claim 1 wherein said virus is FeLV-B.

6. The cell of claim 1 wherein said virus is avian leukosis virus.

7. The cell of claim 1 wherein said virus is NZB virus.

8. The cell of claim 1 wherein said virus is HVJ virus.

9. The cell of claim 1 wherein said portion of said virus is the envelope protein.

10. The cell of claim 1 wherein said cell is selected from the group consisting of the HOS, TE671, HT1080, MV-1-Lu, and human 293 cell lines, and cell lines derived from the HOS, TE671, HT1080, Mv-1-Lu, and human 293 cell lines.

11. The cell of claim 10 wherein said cell line is a human 293 cell line or a cell line derived from a human 293 cell line.

12. A producer cell for producing retroviral vectors resistant to inactivation by human serum, said producer cell being resistant to lysis by human serum and including: (i)a polynucleotide encoding a retroviral envelope proteins, said polynucleotide encoding said envelope protein being obtained from a virus selected from the group consisting of feline endogenous virus RD114, BaEV,SSAV, FeLV-B, NZB virus, avian leukosis virus, and HVJ virus, and (ii) a retroviral vector including a 5' LTR, a 3' LTR, a packaging signal, and at least one polynucleotide encoding a protein or polypeptide of interest, wherein said producer cell does not include the entire viral RNA of feline endogenous virus RD114, BaEV,SSAV, FeLV-B, NZB virus, avian leukosis virus, or HVS virus.

13. The cell of claim 12 wherein said envelope protein is obtained from feline endogenous virus RD114.

14. The cell of claim 12 where said envelope protein is obtained from BaEV.

15. The cell of claim 12 wherein said envelope protein is obtained from SSAV.

16. The cell of claim 12 wherein said envelope protein is obtained from FeLV-B.

17. The cell of claim 12 wherein said envelope protein is obtained from NZB virus.

18. The cell of claim 12 wherein said envelope protein is obtained from avian leukosis virus.

19. The cell of claim 12 wherein said envelope protein is obtained from HVJ virus.

20. A process for producing **retroviral vector particles** resistant to inactivation by human serum, comprising; generating **retroviral vector particles** from the producer cell of claim 12, said **retroviral vector particles** being resistant to inactivation by human serum.

21. A method of producing retroviral vectors resistant to inactivation by human serum, comprising: determining resistance of cells to lysis by human serum; and producing retroviral vectors from cells determined to be resistant to lysis by human serum.

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=> d his

     (FILE 'HOME' ENTERED AT 13:06:04 ON 15 OCT 2006)

     FILE 'USPATFULL' ENTERED AT 13:06:13 ON 15 OCT 2006
                E DOMS ROBERT W/IN
L1           12 S E3
                E HOXIE JAMES A/IN
L2            8 S E3
L3            4 S L2 NOT L1
                E BATES PAUL/IN
L4            6 S E3-E6
L5            5 S L4 NOT (L1 OR L2)
                E HOFFMAN TREVOR L/IN
L6            4 S E3
L7            0 S L6 NOT (L1 OR L2)
                E RUCKER JOSEPH/IN
L8            4 S E3-E5
L9            3 S L8 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 13:10:28 ON 15 OCT 2006
                E DOMS ROBERT W/IN
L10           7 S E1-E2
                E HOXIE J A/IN
L11           6 S E3
L12           4 S L11 NOT L10

     FILE 'MEDLINE' ENTERED AT 13:12:03 ON 15 OCT 2006
                E DOMS R W/AU
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L14         129 S L13 AND (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PA
L15           1 S L14 AND (SEVEN-SPANNING MEMBRANE OR MULTIPLE MEMBRANE OR MULT
L16          75 S L14 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID O
L17           0 S L16 AND (OPIOID OR OSPIN? OR BACTERIORHODOPSIN OR RHODOPSIN O
L18           0 S L16 AND (VIRUS-LIKE PARTICLE OR VLP OR VIRAL PSEUDOTYPE?)
L19          15 S L16 AND PY<1998

     FILE 'USPATFULL' ENTERED AT 13:36:57 ON 15 OCT 2006
L20        3910 S (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PARTICLE?
L21         678 S L20 AND (BACTERIORHODOPSIN OR RHODOPSIN OR OPSIN? OR PORIN? O
L22          17 S L21 AND (VLP?/CLM OR VIRUS-LIKE PARTICLE?/CLM OR RVVP?/CLM OR
L23           0 S L22 AND (BACTERIORHODOPSIN/CLM OR RHODOPSIN/CLM OR OPSIN?/CLM

=> s l20 and (VLP?/clm or virus-like particle?/clm or RVVP?/clm or retroviral vector particle?/clm)
           155 VLP?/CLM
         17990 VIRUS/CLM
        214131 LIKE/CLM
        177693 PARTICLE?/CLM
           232 VIRUS-LIKE PARTICLE?/CLM
                 ((VIRUS(W)LIKE(W)PARTICLE?)/CLM)
             2 RVVP?/CLM
          2090 RETROVIRAL/CLM
         64123 VECTOR/CLM
        177693 PARTICLE?/CLM
            55 RETROVIRAL VECTOR PARTICLE?/CLM
                 ((RETROVIRAL(W)VECTOR(W)PARTICLE?)/CLM)
L24         370 L20 AND (VLP?/CLM OR VIRUS-LIKE PARTICLE?/CLM OR RVVP?/CLM OR
               RETROVIRAL VECTOR PARTICLE?/CLM)

=> s l24 and (CCR5/clm or CXCR4/clm or MCAT?/clm or CXCR2/clm or CXCR3/clm)
           330 CCR5/CLM
           222 CXCR4/CLM
             8 MCAT?/CLM
            77 CXCR2/CLM
            79 CXCR3/CLM
L25           6 L24 AND (CCR5/CLM OR CXCR4/CLM OR MCAT?/CLM OR CXCR2/CLM OR
               CXCR3/CLM)

=> d l25,cbib,clm,1-6

L25  ANSWER 1 OF 6  USPATFULL on STN
2006:247198 Virus-like particles for the induction of autoantibodies.
    Schiller, John T., Silver Spring, MD, UNITED STATES
    Chackerian, Bryce, Chevy Chase, MD, UNITED STATES
    Lowy, Douglas R., Bethesda, MD, UNITED STATES
    US 2006210587 A1 20060921
    APPLICATION: US 2006-415611  A1  20060501 (11)
    PRIORITY: DE 2005-102005020537  20050503
    US 1998-105132P  19981021 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CLM    What is claimed is:
       1. A composition comprising:  a support having an ordered assembly of
       subunits; and  at least one B cell epitope of a tolerogen joined to the
       support so as to form a tolerogen-presenting immunogen, wherein the
       tolerogen-presenting immunogen displays the tolerogen in a regular,
       repetitive array.

       2. The composition of claim 1, wherein the support is a virus-like
       particle, a capsomeric structure, or a bead.

       3. The composition of claim 2, wherein the virus-like particle,
       the capsomeric structure, or the bead comprises a virus capsid protein.

       4. The composition of claim 3, wherein the virus-like particle,
       the capsomeric structure, or the bead comprises a virus capsid protein
       of an icosohedral virus.

       5. The composition of claim 4, wherein the icosohedral virus is one with
       T=7 symmetry.

       6. The composition of claim 5, wherein the icosohedral virus is a
       papillomavirus.

       7. A composition comprising:  a capsomeric structure having a
       symmetrical assembly of capsid proteins; and  at least one B cell
       epitope of a tolerogen joined to the capsomeric structure so as to form
       a tolerogen presenting virus-like particle (VLP), wherein the
       tolerogen presenting VLP displays the tolerogen in an ordered,
       repetitive array.

       8. The composition of claim 7, wherein the capsid protein is a capsid
       protein from a virus selected from the group consisting of
```

papillomaviridae, polyomaviridae, and parvoviridae.

9. The composition of claim 7, wherein the capsid protein is a papillomavirus L1 protein.

10. The composition of claim 7, wherein the capsomeric structure is icosohedral.

11. The composition of claim 1 or claim 7, wherein the tolerogen is joined to the support by a linker.

12. The composition of claim 11, wherein the linker comprises biotin.

13. The composition of claim 1 or claim 7, wherein the tolerogen is selected from the group consisting of a peptide, nucleic acid, carbohydrate, and lipid.

14. The composition of claim 1 or claim 7, wherein the tolerogen is a self antigen.

15. The composition of claim 1 or claim 7, wherein the tolerogen comprises a protein expressed on the surface of a cell.

16. The composition of claim 1 or claim 7, wherein the tolerogen is a protein associated with angiogenesis.

17. The composition of claim 1 or claim 7, wherein the tolerogen is **CCR5**.

18. The composition of claim 1 or claim 7, wherein the tolerogen is Tumor Necrosis Factor α (TNF-α).

19. An isolated complex comprising the composition of claim 1 or claim 7 joined to a cell of the immune system.

20. A pharmaceutical comprising the composition of claim 1 or claim 7.

21. A method of generating antibodies to a tolerogen comprising; identifying a subject in need of antibodies to a tolerogen; and providing to the subject a sufficient amount of the composition of claim 1 or claim 7 to generate antibodies to the tolerogen.

22. The method of claim 21, wherein the tolerogen is a self antigen.

23. A method of identifying agents that generate auto-antibodies comprising: (a) providing the composition of claim 1 or claim 7 to a subject; (b) isolating antibodies from the subject; (c) determining the titer of the antibodies isolated in step (b) that bind to the tolerogen; and (d) identifying the agent by the ability to generate high titer antibodies.

24. A method of inhibiting HIV infection comprising the step of administering the pharmaceutical of claim 20.

25. A method of reducing inflammation comprising administering the pharmaceutical of claim 20.

26. A method of treating chronic viral infection comprising administering the pharmaceutical of claim 20.

27. A method of generating high titer antibodies comprising administering the agent identified by the method of claim 23 to a subject in need thereof.

28. A method of generating monoclonal antibodies to a tolerogen comprising: providing the composition of claim 1 or claim 7 to a subject; and making a hybridoma with a B cell from the subject.

29. A method of enhancing the production of antibodies to a normally immunogenic compound comprising: selecting an antigen that generates a low titer antibody response in a subject; joining this antigen to a modified **VLP** so as to form a conjugated **VLP**, wherein the conjugated **VLP** displays the antigen in a regular repetitive array; and providing the conjugated **VLP** to a subject and thereby generating high titer antibodies.

30. The method of claim 29 wherein the spacing between the antigens is above 50 angstroms.

31. The method of claim 29 wherein the modified **VLP** is joined to the antigen by way of a biotin molecule.

2006:15448 Recombinant measles viruses expressing epitopes of antigens of RNA viruses - use for the preparation of vaccine compositions.
Tangy, Frederic, Les Lilas, FRANCE
Lorin, Clarisse, Paris, FRANCE
Mollet, Lucile, Paris, FRANCE
Delebecque, Frederic, Paris, FRANCE
US 2006013826 A1 20060119
APPLICATION: US 2004-14842 A1 20041220 (11)
PRIORITY: EP 2002-291550 20020620
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. Recombinant mononegavirales virus expressing a heterologous amino acid sequence, said recombinant virus being capable of eliciting a humoral and/or a cellular immune response against said heterologous amino acid sequence including in individuals having pre-existing measles virus immunity.

2. Recombinant measles virus expressing a heterologous amino acid sequence derived from an antigen of a determined RNA virus, said recombinant measles virus being capable of eliciting a humoral and/or a cellular immune response against measles virus or against said RNA virus or against both measles virus and against said RNA virus.

3. Recombinant measles virus according to claim 2 which is the product of the expression in a cell of a recombinant nucleotide sequence comprising acDNA molecule encoding the full length antigenomic (+) RNA of the measles virus (MV) and further comprising, recombined with said cDNA molecule, a sequence encoding an heterologous amino acid sequence of a determined retrovirus or flavivirus.

4. Recombinant measles virus according to claims 1 or 2, which is derived from the Schwarz vaccine strain or from the Edmonston strain.

5. Recombinant measles virus according to anyone of claims 2 to 4, which is rescued from helper cells transfected with a recombinant nucleotide sequence which comprises cDNA encoding the nucleotid sequence of full length antigenomic (+) RNA of the measles virus, said cDNA being recombined with a nucleotide sequence encoding a retroviral orflaviviral heterologous amino acid sequence, and said recombinant nucleotide sequence complying with the rule of six.

6. Recombinant measles virus according to anyone of claims 2 to 5, wherein the nucleotide sequence is comprised within the EdB-tag virus vector recombined with the ATU inserted in a position of the EdB-tag vector taking advantage of the gradient of the viral genome to allow various levels of expression of the transgenic nucleotide sequence encoding the heterologous amino acid sequence inserted in said ATU.

7. Recombinant measles virus according to anyone of claims 2 to 5, which is the product of the expression of a recombinant nucleotide sequence comprising a cDNA molecule which encodes the nucleotide sequence of the full length antigenomic (+) RNA of a measles virus (MV) originating from an approved vaccine strain, wherein said cDNA molecule is recombined with a heterologous nucleotide sequence encoding a heterologous amino acid sequence derived from an antigen of a determined heterologous retrovirus or flavivirus.

8. Recombinant measles virus according to claim 7, wherein the cDNA molecule comprises the insert contained in plasmid pTM-MVSchw deposited on Jun. 12, 2002 under No. 1-2889, wherein said insert encodes the nucleotide sequence of the full length antigenomic (+) RNA strand of the measles virus.

9. Recombinant measles virus according to claim 7 or 8, wherein the recombinant nucleotide sequence is derived from the insert contained in plasmid pTM-MVSchw2-gfp deposited on Jun. 12, 2002 under I-2890 (CNCM), wherein the sequence of the gfp gene is substituted for a sequence encoding a determined amino acid sequence.

10. Recombinant measles virus according to anyone of claims 7 to 9, wherein the cDNA molecule is selected among the following sequences: nucleotide sequence extending from nucleotide 83 to nucleotide 15977 of FIG. 11 nucleotide sequence extending from nucleotide 29 to nucleotide 15977 of FIG. 11 nucleotide sequence extending from nucleotide 29 to nucleotide 16202 of FIG. 11 nucleotide sequence extending from nucleotide 26 to nucleotide 15977 of FIG. 11 nucleotide sequence extending from nucleotide 26 to nucleotide 16202 of FIG. 11 nucleotide sequence extending from nucleotide 9 to nucleotide 15977 of FIG. 11 nucleotide sequence extending from nucleotide 9 to nucleotide 16202 of FIG. 11

11. Recombinant measles virus according to anyone of claims 2 to 10, wherein the heterologous amino acid sequence is derived from an antigen of a retrovirus selected among HIV retroviruses, or from an antigen of a flavivirus.

12. Recombinant measles virus according to anyone of claims 2 to 9, wherein the heterologous amino acid sequence is derived from an antigen of the Yellow Fever Virus or the West Nile Virus.

13. Recombinant measles virus according to anyone of claims 2 to 11, wherein the heterologous amino acid sequence is derived from an envelope antigen of the HIV retrovirus.

14. Recombinant measles virus according to claim 13, wherein the heterologous amino acid sequence is a recombinant gp160 or a recombinant gp120 antigens of HIV-1.

15. Recombinant measles virus according to claim 14, wherein the V1, V2 and/or V3 loops of the gp120 antigen are deleted or deleted in part, individually or in combination in such a way that conserved epitopes are exposed on the obtained recombinant gp120 antigen.

16. Recombinant measles virus according to claim 15, wherein the V1, V2 and/or V3 loops of the gp120 antigen are substituted or substituted in part, individually or in combination in such a way that conserved epitopes are exposed on the obtained recombinant gp120 antigen.

17. Recombinant measles virus according to claim 16, wherein the V3 loop is substituted for a gp41 epitope such as AAELDKWASAA (SEQ ID NO: 8).

18. Recombinant measles virus according to anyone of claims 11 to 16, wherein the heterologous amino acid sequence isgp160 ΔV3, gp160 ΔV1V2, gp160 ΔV1V2V3, gp140 ΔV3, gp140 ΔV1V2, gp140 ΔV1V2V3.

19. Recombinant measles virus according to anyone of claims 2 to 12, wherein the amino acid sequence is derived from an antigen of the Yellow Fever virus selected among the envelope (Env) or the NS1 proteins or immunogenic mutants thereof.

20. Recombinant measles virus according to anyone of claims 2 to 12, wherein the amino acid sequence is derived from an antigen of the West Nile virus selected among the envelope (E), premembrane(preM) or immunogenic mutants thereof.

21. Recombinant measles virus according to anyone of claims 2 to 12, capable of inducing protection against heterologous **VLP** or pM/E proteins.

22. Recombinant measles virus according to anyone of claims 2 to 21, which elicits a humoral and/or a cellular immune response in an animal model susceptible to measles virus.

23. Recombinant measles virus according to anyone of claims 2 to 22, which elicits neutralizing antibodies against the heterologous amino acid sequence in a mammalian animal model susceptible to measles virus.

24. Recombinant measles virus according to anyone of claims 2 to 23 wherein the heterologous amino acid sequence is derived from an envelope proteins of HIV-1 and which elicits antibodies capable of neutralizing a primary HIV isolate when tested on indicator cells such as P4-**CCR5** cells.

25. Recombinant measles virus according to claims 2 to 24 which elicits neutralizing antibodies against the heterologous amino acid sequence in a mammal.

26. Recombinant measles virus vector comprising a replicon comprising (i) a cDNA sequence encoding the full length antigenomic (+) RNA of a measles virus operatively linked to (ii) expression control sequences and (iii) a heterologous DNA sequence coding for a determined heterologous amino acid sequence, said heterologous DNA sequence being cloned in said replicon in conditions allowing its expression and said replicon having a total number of nucleotides which complies with the rule of six.

27. Recombinant measles virus vector according to claim 26, wherein the heterologous DNA sequence is cloned within an Additional Transcription Unit (ATU) inserted in the cDNA corresponding to the antigenomic RNA of measles virus.

28. Recombinant measles virus vector according to claim 26, wherein the cloning site of the ATU is chosen upstream from the N gene of the MV

29. Recombinant measles virus vector according to claim 26, wherein the cloning site of the ATU is chosen between the P and M genes of the MV virus.

30. Recombinant measles virus vector according to claim 26, wherein the cloning site of the ATU is between the H and L genes of the MV virus.

31. Recombinant measles virus vector according to anyone of claims 26 to 29, wherein the heterologous DNA sequence is expressed as a fusion protein with one of the MV proteins.

32. Recombinant measles virus vector according to anyone of claims 26 to 29, wherein the heterologous DNA sequence is not expressed as a fusion protein with one of the MV proteins.

33. Recombinant measles virus vector according to anyone of claims 26 to 29 wherein the heterologous DNA sequence encodes a retroviral amino acid sequence.

34. Recombinant measles virus vector according to anyone of claims 26 to 32 wherein the heterologous DNA sequence encodes a retroviral amino acid sequence derived from an antigen of a retrovirus selected among HIV retroviruses.

35. Recombinant measles virus vector according to anyone of claims 26 to 32 wherein the heterologous DNA sequence encodes a retroviral amino acid sequence derived from an antigen of a retrovirus selected among flaviviruses.

36. Recombinant measles virus vector according to anyone of claims 26 to 33 wherein the heterologous DNA sequence encodes a retroviral amino acid sequence derived from an envelope antigen of an HIV retrovirus.

37. Recombinant measles virus vector according to anyone of claims 26 to 33 wherein the heterologous DNA sequence encodes a retroviral amino acid sequence selected among the gp160, the gp120 orgp41 of HIV-1, or the gp140 of HIV-1, or a mutated version of said antigens.

38. Recombinant measles virus vector according to anyone of claims 26 to 36, wherein the mutated antigen enables exposition of neutralizing epitopes.

39. Recombinant measles virus vector according to anyone of claims 26 to 37, wherein the heterologous DNA sequence encodes gp160AV3, gp160 ΔV1V2, gp160 ΔV1V2V3, gp140 ΔV3, gp140 ΔV1V2, gp140 ΔV1V2V3.

40. Recombinant measles virus vector according to anyone of claims 25 to 32 wherein the heterologous DNA sequence encodes a flaviviral amino acid sequence derived from an antigen of the Yellow Fever virus or the West Nile virus.

41. Recombinant measles virus vector according to anyone of claims 26 to 40 wherein the nucleotide sequence comprising cDNA resulting from reverse transcription of the antigenic RNA of measles virus, originates from a measles virus strain approved for vaccination.

42. Recombinant measles virus vector according to claim 41, wherein the measles virus strain is the Schwarz strain.

43. Recombinant measles virus vector according to claim 42, wherein the cDNA encoding the full length antigenomic (+) RNA of the measles virus and the expression control sequence are derived from pTM-MVSchw deposited at the CNCM under No. 1-2889.

44. Recombinant measles virus vector according to anyone of claims 26 to 42, which is a plasmid.

45. Recombinant measles virus vector according to anyone of claims 26 to 44 wherein the replicon is designed according to the map of FIG. 2 wherein insert represents the heterologous DNA sequence.

46. Recombinant measles virus vector according to anyone of claims 26 to 45, wherein the heterologous DNA sequence is selected among YFV17D204.

47. Recombinant measles virus vector according to anyone of claims 26 to 45, wherein the heterologous DNA sequence is selected among the neurovirulent strain IS-98-ST1.

48. Recombinant measles virus vector according to claim 43, which is selected among the following vectors deposited with the CNCM

pMV2 (EdB)gp160 [delta] V3HIV89. 6P	CNCM 1-2883
pMV2 (EdB) gp160HIV89. 6P	CNCM 1-2884
pMV2 (EdB) gp140HIV89. 6P	CNCM 1-2885
pMV3 (EdB)gp140 [delta] V3HIV89. 6P	CNCM 1-2886
pMV2(EdB)-NS1YFV17D	CNCM I-2887
pMV2 (EdB)-EnvYFV17D	CNCM 1-2888.
pTM-MVSchw2-Es(WNV)	CNCM I-3033
pTM-MVSchw2-GFPbis-	CNCM 1-3034
pTM-MVSchw2-p17p24[delta] myr(HIVB)	CNCM 1-3035
pTM-MVSchw3-Tat(HIV89-6p)	CNCM I-3036
pTM-MVschw3-GFP	CNCM 1-3037
pTM-MVSchw2-Es (YFV)	CNCM 1-3038
pTM-MVSchw2-gp140 [delta] V1 V2 V3(HIV89-6)	CNCM 1-3054
pTM-MVSchw2-gp140 [delta] V3(HIV89-6)	CNMC I-3055
pTM-MVSchw2-gp160 [dela] V1 V2 V3(HIV89-6)	CNCM 1-3056
pTM-MVSchw2-gp160 [delta] V1 V2(HIV89-6)	CNCM 1-3057
pTM-MVSchw2-Gag SIV239 p17-p24 [delta] myr-3-gp 140 (HIV89-6)	CNCM I-3058.

49. A rescue system for the assembly of recombinant measles virus expressing a heterologous amino acid sequence, which comprises a determined cell transfected with a recombinant measles virus vector according to anyone of claims 26 to 47, and a determined helper cell recombined with at least one vector suitable for expression of T7 RNA polymerase and expression of the N, P and L proteins of the measles virus.

50. An immunogenic composition comprising a recombinant virus according to anyone of claims 1 to 26, or a recombinant vector according to anyone of claims to 48.

51. A vaccine composition comprising a recombinant virus according to anyone of claims 1 to 26, or a recombinant vector according to anyone of claims to 48.

L25 ANSWER 3 OF 6 USPATFULL on STN

2004:291718 **Virus-like particles** for the induction of autoantibodies.

Schiller, John T., Silver Spring, MD, UNITED STATES
Chackerian, Bryce, Chevy Chase, MD, UNITED STATES
Lowy, Douglas R., Bethesda, MD, UNITED STATES
US 2004228798 A1 20041118
APPLICATION: US 2004-867119 A1 20040614 (10)
PRIORITY: US 1998-105132P 19981021 (60)
DOCUMENT TYPE: Utility; APPLICATION.

CLM What is claimed is:

1. A composition comprising: a support having an ordered assembly of subunits; and at least one B cell epitope of a tolerogen joined to the support so as to form a tolerogen-presenting immunogen, wherein the tolerogen-presenting immunogen displays the tolerogen in a regular, repetitive array.

2. The composition of claim 1, wherein the support is a **virus-like particle**, a capsomeric structure, or a bead.

3. The composition of claim 2, wherein the **virus-like particle**, the capsomeric structure, or the bead comprises a virus capsid protein.

4. The composition of claim 3, wherein the **virus-like particle**, the capsomeric structure, or the bead comprises a virus capsid protein of an icosohedral virus.

5. The composition of claim 4, wherein the icosohedral virus is one with T=7 symmetry.

6. The composition of claim 5, wherein the icosohedral virus is a papillomavirus.

7. A composition comprising: a capsomeric structure having a symmetrical assembly of capsid proteins; and at least one B cell epitope of a tolerogen joined to the capsomeric structure so as to form a tolerogen presenting **virus-like particle (VLP)**, wherein the tolerogen presenting **VLP** displays the tolerogen in an ordered, repetitive array.

8. The composition of claim 7, wherein the capsid protein is a capsid protein from a virus selected from the group consisting of papillomavirinae, polyomavirinae, and parvoviridae.

9. The composition of claim 7, wherein the capsid protein is a papillomavirus L1 protein.

10. The composition of claim 7, wherein the capsomeric structure is icosohedral.

11. The composition of claim 1 or claim 7, wherein the tolerogen is joined to the support by a linker.

12. The composition of claim 11, wherein the linker comprises biotin.

13. The composition of claim 1 or claim 7, wherein the tolerogen is selected from the group consisting of a peptide, nucleic acid, carbohydrate, and lipid.

14. The composition of claim 1 or claim 7, wherein the tolerogen is a self antigen.

15. The composition of claim 1 or claim 7, wherein the tolerogen comprises a protein expressed on the surface of a cell.

16. The composition of claim 1 or claim 7, wherein the tolerogen is a protein associated with angiogenesis.

17. The composition of claim 1 or claim 7, wherein the tolerogen is **CCR5**.

18. The composition of claim 1 or claim 7, wherein the tolerogen is Tumor Necrosis Factor α (TNF-α).

19. An isolated complex comprising the composition of claim 1 or claim 7 joined to a cell of the immune system.

20. A pharmaceutical comprising the composition of claim 1 or claim 7.

21. A method of generating antibodies to a tolerogen comprising; identifying a subject in need of antibodies to a tolerogen; and providing to the subject a sufficient amount of the composition of claim 1 or claim 7 to generate antibodies to the tolerogen.

22. The method of claim 21, wherein the tolerogen is a self antigen.

23. A method of identifying agents that generate auto-antibodies comprising: (a) providing the composition of claim 1 or claim 7 to a subject; (b) isolating antibodies from the subject; (c) determining the titer of the antibodies isolated in step (b) that bind to the tolerogen; and (d) identifying the agent by the ability to generate high titer antibodies.

24. A method of inhibiting HIV infection comprising the step of administering the pharmaceutical of claim 20.

25. A method of reducing inflammation comprising administering the pharmaceutical of claim 20.

26. A method of treating chronic viral infection comprising administering the pharmaceutical of claim 20.

27. A method of generating high titer antibodies comprising administering the agent identified by the method of claim 23 to a subject in need thereof.

28. A method of generating monoclonal antibodies to a tolerogen comprising: providing the composition of claim 1 or claim 7 to a subject; and making a hybridoma with a B cell from the subject.

29. A method of enhancing the production of antibodies to a normally immunogenic compound comprising: selecting an antigen that generates a low titer antibody response in a subject; joining this antigen to a modified **VLP** so as to form a conjugated **VLP**, wherein the conjugated **VLP** displays the antigen in a regular repetitive array; and providing the conjugated **VLP** to a subject and thereby generating high titer antibodies.

30. The method of claim 29 wherein the spacing between the antigens is above 50 angstroms.

31. The method of claim 29 wherein the modified **VLP** is joined to the antigen by way of a biotin molecule.

L25 ANSWER 4 OF 6 USPATFULL on STN
2003:105842 **Virus-like particles** for the induction of autoantibodies.
Schiller, John T., Silver Spring, MD, UNITED STATES
Chackerian, Bryce, Chevy Chase, MD, UNITED STATES

US 2003072753 A1 20030417
APPLICATION: US 2002-253443 A1 20020924 (10)
PRIORITY: US 1998-105132P 19981021 (60)
DOCUMENT TYPE: Utility; APPLICATION.

CLM What is claimed is:

1. A composition comprising: a support having an ordered assembly of subunits; and at least one B cell epitope of a tolerogen joined to the support so as to form a tolerogen-presenting immunogen, wherein the tolerogen-presenting immunogen displays the tolerogen in a regular, repetitive array.

2. The composition of claim 1, wherein the support is a **virus-like particle**, a capsomeric structure, or a bead.

3. The composition of claim 2, wherein the **virus-like particle**, the capsomeric structure, or the bead comprises a virus capsid protein.

4. The composition of claim 3, wherein the **virus-like particle**, the capsomeric structure, or the bead comprises a virus capsid protein of an icosohedral virus.

5. The composition of claim 4, wherein the icosohedral virus is one with T=7 symmetry.

6. The composition of claim 5, wherein the icosohedral virus is a papillomavirus.

7. A composition comprising: a capsomeric structure having a symmetrical assembly of capsid proteins; and at least one B cell epitope of a tolerogen joined to the capsomeric structure so as to form a tolerogen presenting **virus-like particle** (**VLP**), wherein the tolerogen presenting **VLP** displays the tolerogen in an ordered, repetitive array.

8. The composition of claim 7, wherein the capsid protein is a capsid protein from a virus selected from the group consisting of papillomavirinae, polyomavirinae, and parvoviridae.

9. The composition of claim 7, wherein the capsid protein is a papillomavirus L1 protein.

10. The composition of claim 7, wherein the capsomeric structure is icosohedral.

11. The composition of claim 1 or claim 7, wherein the tolerogen is joined to the support by a linker.

12. The composition of claim 11, wherein the linker comprises biotin.

13. The composition of claim 1 or claim 7, wherein the tolerogen is selected from the group consisting of a peptide, nucleic acid, carbohydrate, and lipid.

14. The composition of claim 1 or claim 7, wherein the tolerogen is a self antigen.

15. The composition of claim 1 or claim 7, wherein the tolerogen comprises a protein expressed on the surface of a cell.

16. The composition of claim 1 or claim 7, wherein the tolerogen is a protein associated with angiogenesis.

17. The composition of claim 1 or claim 7, wherein the tolerogen is **CCR5**.

18. The composition of claim 1 or claim 7, wherein the tolerogen is Tumor Necrosis Factor a (TNF-α).

19. An isolated complex comprising the composition of claim 1 or claim 7 joined to a cell of the immune system.

20. A pharmaceutical comprising the composition of claim 1 or claim 7.

21. A method of generating antibodies to a tolerogen comprising; identifying a subject in need of antibodies to a tolerogen; and providing to the subject a sufficient amount of the composition of claim 1 or claim 7 to generate antibodies to the tolerogen.

22. The method of claim 21, wherein the tolerogen is a self antigen.

23. A method of identifying agents that generate auto-antibodies comprising: (a) providing the composition of claim 1 or claim 7 to a

the titer of the antibodies isolated in step (b) that bind to the tolerogen; and (d) identifying the agent by the ability to generate high titer antibodies.

24. A method of inhibiting HIV infection comprising the step of administering the pharmaceutical of claim 20.

25. A method of reducing inflammation comprising administering the pharmaceutical of claim 20.

26. A method of treating chronic viral infection comprising administering the pharmaceutical of claim 20.

27. A method of generating high titer antibodies comprising administering the agent identified by the method of claim 23 to a subject in need thereof.

28. A method of generating monoclonal antibodies to a tolerogen comprising: providing the composition of claim 1 or claim 7 to a subject; and making a hybridoma with a B cell from the subject.

29. A method of enhancing the production of antibodies to a normally immunogenic compound comprising: selecting an antigen that generates a low titer antibody response in a subject; joining this antigen to a modified **VLP** so as to form a conjugated **VLP**, wherein the conjugated **VLP** displays the antigen in a regular repetitive array; and providing the conjugated **VLP** to a subject and thereby generating high titer antibodies.

30. The method of claim 29 wherein the spacing between the antigens is above 50 angstroms.

31. The method of claim 29 wherein the modified **VLP** is joined to the antigen by way of a biotin molecule.

L25 ANSWER 5 OF 6 USPATFULL on STN
2003:76924 Stabilized viral envelope proteins and uses thereof.
Binley, James M., Brooklyn, NY, UNITED STATES
Schuelke, Norbert, New City, NY, UNITED STATES
Olson, William C., Ossining, NY, UNITED STATES
Maddon, Paul J., Scarsdale, NY, UNITED STATES
Moore, John P., New York, NY, UNITED STATES
Progenics Pharmaceuticals, Inc., Aaron Diamonds AIDS Research Center (ADARC) (U.S. corporation)
US 2003052839 A1 20030320
APPLICATION: US 2001-32162 A1 20011221 (10)
DOCUMENT TYPE: Utility; APPLICATION.

CLM What is claimed is:

1. An isolated nucleic acid which comprises a nucleotide segment having a sequence encoding a complex comprising a viral surface protein and a corresponding viral transmembrane protein wherein the complex contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and the viral transmembrane protein.

2. The isolated nucleic acid of claim 1, wherein the virus is a lentivirus.

3. The isolated nucleic acid of claim 1, wherein the virus is the human immunodeficiency virus.

4. The isolated nucleic acid of claim 3, wherein the human immunodeficiency virus is a primary isolate.

5. The isolated nucleic acid of claim 3, wherein the human immunodeficiency virus is HIV-1_{JR-FL}, HIV-1_{DH123}, HIV-1_{Gun-1}, HIV-1_{89}.6, or HIV-1_{HXB2}.

6. The isolated nucleic acid of claim 3, wherein the viral surface protein is gp120 or a modified form of gp120, wherein the modification alters the immunogenicity of the molecule relative to wild type gp120.

7. The isolated nucleic acid of claim 6, wherein the modified gp120 molecule is characterized by the absence of one or more variable loops present in wild type gp120.

8. The isolated nucleic acid of claim 7, wherein the variable loop comprises V1, V2, or V3.

9. The isolated nucleic acid of any one of claims 68, wherein the modified gp120 molecule is characterized by the absence or presence of

wild type gp120.

10. The isolated nucleic acid of claim 9, wherein one or more canonical glycosylation sites are absent from the V1V2 region of the gp120 molecule.

11. The isolated nucleic acid of any one of claims 3-10, wherein the transmembrane protein is gp41 or a modified form of gp41, wherein the modification alters the immunogenicity of the molecule relative to wildtype gp41.

12. The isolated nucleic acid of claim 11, wherein the transmembrane protein is the gp41 ectodomain.

13. The isolated nucleic acid of claim 11 or 12, wherein the transmembrane protein is modified by the absence or presence of one or more canonical glycosylation sites absent or present in the wild type gp120.

14. The isolated nucleic acid of any one of claims 1-13, wherein the stabilization of the complex is achieved by one or more cysteine-cysteine bonds that are formed between the surface and transmembrane proteins and that are not present in the corresponding wildtype complex.

15. The isolated nucleic acid of claim 14, wherein one or more amino acids which are adjacent to or which contain an atom within 5 angstroms of an introduced cysteine are mutated to a noncysteine residue.

16. The isolated nucleic acid of claim 14 or 15, wherein one or more cysteines in gp120 or modified form of gp120 are disulfide linked to one or more cysteines in gp41 or modified form of gp41.

17. The isolated nucleic acid of claim 16, wherein a cysteine in the C5 region of gp120 or modified form of gp120 is disulfide linked to a cysteine in the ectodomain of gp41.

18. The isolated nucleic acid of claim 16, wherein the disulfide bond is formed between a cysteine introduced by an A492C mutation in gp120 and a T596C mutation in gp41.

19. The isolated nucleic acid molecule of claim 1 which is cDNA.

20. The isolated nucleic acid molecule of claim 1 which is genomic DNA.

21. The isolated nucleic acid molecule of claim 1 which is RNA.

22. A replicable vector comprising the nucleic acid of claim 1.

23. A plasmid, cosmid, λ phage or YAC containing the nucleic acid of claim 1.

24. The plasmid of claim 23 designated PPI4.

25. A host cell containing the vector of claim 22.

26. The cell of claim 25 which is a eukaryotic cell.

27. The cell of claim 25 which is a bacterial cell.

28. A vaccine which comprises the isolated nucleic acid of claim 1.

29. A vaccine which comprises a therapeutically effective amount of the nucleic acid of claim 1.

30. A vaccine which comprises a therapeutically effective amount of the protein encoded by the nucleic acid of claim 1.

31. A method of treating a viral disease which comprises immunizing a virally infected subject with the vaccine of claim 29 or 30 or a combination thereof, thereby treating the subject.

32. A vaccine which comprises a prophylactically effective amount of the nucleic acid of claim 1.

33. A vaccine which comprises a prophylactically effective amount of the protein encoded by the nucleic acid of claim 1.

34. A method of reducing the likelihood of a subject becoming infected with a virus comprising administering the vaccine of claim 32 or 33 or a combination thereof, thereby reducing the likelihood of the subject becoming infected with the virus.

35. A vaccine comprising the nucleic acid of any one of claims 3-18.

36. A vaccine which comprises a therapeutically effective amount of the nucleic acid of any one of claims 3-18.

37. A vaccine which comprises a therapeutically effective amount of the protein encoded by the nucleic acid of any one of claims 3-18.

38. A method of treating an HIV-1 infected subject which comprises immunizing the subject with the vaccine of claim 36 or 37 or a combination thereof, thereby treating the subject.

39. A vaccine which comprises a prophylactically effective amount of the nucleic acid of any one of claims 3-18.

40. A vaccine which comprises a prophylactically effective amount of the protein encoded by the nucleic acid of any one of claims 3-18.

41. A method of reducing the likelihood of a subject becoming infected with HIV-1 comprising administering the vaccine of claim 39 or 40 or a combination thereof, thereby reducing the likelihood of the subject becoming infected with HIV-1.

42. The vaccine of claim 35, wherein the vaccine comprises a recombinant subunit protein, a DNA plasmid, a replicating viral vector, a non-replicating viral vector, or a combination thereof.

43. A method of reducing the severity of HIV-1 disease in a subject comprising administering the vaccine of claim 39 or 40 or a combination thereof, prior to exposure of the subject to HIV-1, thereby reducing the severity of HIV-1 disease or AIDS in the subject upon subsequent exposure to HIV-1.

44. A viral envelope protein comprising a viral surface protein and a corresponding viral transmembrane protein wherein the viral envelope protein contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and transmembrane protein.

45. A complex comprising a viral surface protein and a viral transmembrane protein, wherein the complex contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and transmembrane protein.

46. A mutant HIV-1 envelope protein which is encoded by the nucleic acid of any one of claims 3-18.

47. The protein of claim 44 or complex of claim 45 which is linked to at least one other protein or protein fragment to form a fusion protein.

48. A purified protein of any one of claims 44-46.

49. A vaccine which comprises a therapeutically effective amount of the protein of claim 44 or the complex of claim 45.

50. A vaccine which comprises a prophylactically effective amount of the protein of claim 44 or the complex of claim 45.

51. A method of stimulating or enhancing in a subject production of antibodies which recognize the protein of claim 44 or complex of claim 45.

52. An antibody, antibody chain, fragment or derivative thereof isolated or identified using the viral envelope protein encoded by the recombinant nucleic acid of claim 1.

53. The antibody of claim 52, wherein the antibody is of the IgM, IgA, IgE or IgG class or subclasses thereof.

54. The antibody fragment of claim 52 which includes but is not limited to Fab, Fab' (Fab')2, Fv and single chain antibodies.

55. The isolated antibody light chain of the antibody of claim 52, or fragment or oligomer thereof.

56. The isolated antibody heavy chain of the antibody of claim 52, or fragment or oligomer thereof.

57. One or more complementarity determining regions of the antibody of claim 52.

58. The antibody of claim 52 which is derivatized such as by the addition of a fluorescent moiety, a radionuclide, an enzyme, a toxin, or an affinity ligand such as biotin.

59. The antibody of claim 52 wherein the antibody is a human antibody.

60. The antibody of claim 52 or 59, wherein the antibody is a monoclonal antibody.

61. The antibody of claim 52, wherein the antibody is a humanized antibody.

62. The antibody of claim 52 or any one of claims 59-61, wherein the viral envelope protein is derived from HIV-1.

63. An isolated nucleic acid molecule encoding the antibody of claim 52 or any one of claims 59-61, wherein the nucleic acid molecule is RNA, genomic DNA or cDNA.

64. The isolated nucleic acid of claim 63, wherein the viral envelope protein is derived from HIV-1.

65. An agent capable of inhibiting the binding of the antibody of claim 52.

66. A method of reducing the likelihood of an HIV-1-exposed subject from becoming infected with HIV-1 comprising administering the antibody of claim 62 or the isolated nucleic acid of claim 64, thereby reducing the likelihood of the HIV-1 exposed subject from becoming infected with HIV-1.

67. A method of treating a subject infected with HIV-1 comprising administering the antibody of claim 62 or the isolated nucleic acid of claim 64, thereby treating the subject.

68. An agent capable of binding the mutant viral envelope protein encoded by the recombinant nucleic acid molecule of claim 1.

69. The agent of claim 68 which inhibits viral infection.

70. The agent of claim 69, wherein the viral envelope protein is derived from HIV-1.

71. A method for determining whether a compound is capable of inhibiting a viral infection comprising: (A) contacting an appropriate concentration of the compound with the mutant viral envelope protein encoded by the nucleic acid of claim 1 under conditions permitting binding of the compound to said protein; (B) contacting the resulting complex with a reporter molecule under conditions that permit binding of the reporter molecule to the mutant viral envelope protein in the absence of the compound; (C) measuring the amount of bound reporter molecule; and (D) comparing the amount of bound reporter molecule in step (c) with the amount determined in the absence of the compound, a decrease in the amount indicating that the compound is capable of inhibiting infection by the virus.

72. The method of claim 71, wherein the reporter molecule is an antibody or derivative thereof.

73. The method of claim 71, wherein the reporter molecule comprises one or more host cell viral receptors or molecular mimics thereof.

74. A method for determining whether a compound is capable of inhibiting a viral infection which comprises: (a) contacting an appropriate concentration of the compound with a host cell viral receptor or molecular mimic thereof under conditions that permit binding of the compound and receptor or receptor mimic in the absence of the compound; (b) contacting the resulting complex with the mutant viral envelope protein encoded by the recombinant nucleic acid of claim 1 under conditions that permit binding of the envelope protein and receptor or receptor mimic in the absence of the compound; (c) measuring the amount of binding of envelope protein to receptor or receptor mimic; (d) comparing the amount of binding determined in step (c) with the amount determined in the absence of the compound, a decrease in the amount indicating that the compound is capable of inhibiting infection by the virus.

75. The method of any one of claims 71-74 wherein the virus is HIV-1.

76. The method of claim 71 or 72, wherein the host cell viral receptor is CD4, **CCR5**, **CXCR4** or combinations or molecular mimics thereof.

77. The method of any one of claims 71-76, wherein the compound was not

previously known.

78. A compound determined to be capable of inhibiting a viral infection by the method of any one of claims 71-76.

79. A pharmaceutical composition comprising an amount of the compound effective to inhibit viral infection determined by the method of any one of claims 71-76 to be capable of inhibiting viral infection and a pharmaceutically acceptable carrier.

80. The pharmaceutical composition of claim 79, wherein the viral infection is HIV-1 infection.

81. A viral envelope protein comprising a viral surface protein and a corresponding viral transmembrane protein wherein the viral envelope protein contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and transmembrane protein, wherein the surface protein and transmembrane protein are encoded by different nucleic acids.

82. A complex comprising a viral surface protein and a corresponding viral transmembrane protein of a viral envelope protein wherein the viral envelope protein contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and transmembrane protein, wherein the surface protein and transmembrane protein are encoded by different nucleic acids.

83. An antibody which binds to the protein of claim 44 or the complex of claim 45 but does not cross react with the individual monomeric surface protein or the individual monomeric transmembrane protein.

84. The antibody of claim 83 capable of binding to the HIV-1 virus.

85. A **virus-like particle** which comprises the complex of claim 45.

86. The **virus-like particle** of claim 85, further comprising an immunodeficiency virus gag protein.

L25 ANSWER 6 OF 6 USPATFULL on STN
2002:156700 **Virus-like particles** for the induction of autoantibodies.
Schiller, John T., Silver Spring, MD, UNITED STATES
Chackerian, Bryce, Chevy Chase, MD, UNITED STATES
Lowy, Douglas R., Bethesda, MD, UNITED STATES
US 2002081295 A1 20020627
APPLICATION: US 2001-835124 A1 20010413 (9)
PRIORITY: US 1998-105132P 19981021 (60)
DOCUMENT TYPE: Utility; APPLICATION.

CLM What is claimed is:

1. A composition comprising: a support having an ordered assembly of subunits; and at least one B cell epitope of a tolerogen joined to the support so as to form a tolerogen-presenting immunogen, wherein the tolerogen-presenting immunogen displays the tolerogen in a regular, repetitive array.

2. The composition of claim 1, wherein the support is a **virus-like particle**, a capsomeric structure, or a bead.

3. The composition of claim 2, wherein the **virus-like particle**, the capsomeric structure, or the bead comprises a virus capsid protein.

4. The composition of claim 3, wherein the **virus-like particle**, the capsomeric structure, or the bead comprises a virus capsid protein of an icosohedral virus.

5. The composition of claim 4, wherein the icosohedral virus is one with T=7 symmetry.

6. The composition of claim 5, wherein the icosohedral virus is a papillomavirus.

7. A composition comprising: a capsomeric structure having a symmetrical assembly of capsid proteins; and at least one B cell epitope of a tolerogen joined to the capsomeric structure so as to form a tolerogen presenting **virus-like particle (VLP)**, wherein the tolerogen presenting **VLP** displays the tolerogen in an ordered, repetitive array.

8. The composition of claim 7, wherein the capsid protein is a capsid protein from a virus selected from the group consisting of papillomavirinae, polyomavirinae, and parvoviridae.

9. The composition of claim 7, wherein the capsid protein is a papillomavirus L1 protein.

10. The composition of claim 7, wherein the capsomeric structure is icosohedral.

11. The composition of claim 1 or claim 7, wherein the tolerogen is joined to the support by a linker.

12. The composition of claim 11, wherein the linker comprises biotin.

13. The composition of claim 1 or claim 7, wherein the tolerogen is selected from the group consisting of a peptide, nucleic acid, carbohydrate, and lipid.

14. The composition of claim 1 or claim 7, wherein the tolerogen is a self antigen.

15. The composition of claim 1 or claim 7, wherein the tolerogen comprises a protein expressed on the surface of a cell.

16. The composition of claim 1 or claim 7, wherein the tolerogen is a protein associated with angiogenesis.

17. The composition of claim 1 or claim 7, wherein the tolerogen is **CCR5**.

18. The composition of claim 1 or claim 7, wherein the tolerogen is Tumor Necrosis Factor a (TNF-α).

19. An isolated complex comprising the composition of claim 1 or claim 7 joined to a cell of the immune system.

20. A pharmaceutical comprising the composition of claim 1 or claim 7.

21. A method of generating antibodies to a tolerogen comprising; identifying a subject in need of antibodies to a tolerogen; and providing to the subject a sufficient amount of the composition of claim 1 or claim 7 to generate antibodies to the tolerogen.

22. The method of claim 21, wherein the tolerogen is a self antigen.

23. A method of identifying agents that generate auto-antibodies comprising: (a) providing the composition of claim 1 or claim 7 to a subject; (b) isolating antibodies from the subject; (c) determining the titer of the antibodies isolated in step (b) that bind to the tolerogen; and 1 (d) identifying the agent by the ability to generate high titer antibodies.

24. A method of inhibiting HIV infection comprising the step of administering the pharmaceutical of claim 20.

25. A method of reducing inflammation comprising administering the pharmaceutical of claim 20.

26. A method of treating chronic viral infection comprising administering the pharmaceutical of claim 20.

27. A method of generating high titer antibodies comprising administering the agent identified by the method of claim 23 to a subject in need thereof.

28. A method of generating monoclonal antibodies to a tolerogen comprising: providing the composition of claim 1 or claim 7 to a subject; and making a hybridoma with a B cell from the subject.

29. A method of enhancing the production of antibodies to a normally immunogenic compound comprising: selecting an antigen that generates a low titer antibody response in a subject; joining this antigen to a modified **VLP** so as to form a conjugated **VLP**, wherein the conjugated **VLP** displays the antigen in a regular repetitive array; and providing the conjugated **VLP** to a subject and thereby generating high titer antibodies.

30. The method of claim 29 wherein the spacing between the antigens is above 50 angstroms.

31. The method of claim 29 wherein the modified **VLP** is joined to the antigen by way of a biotin molecule.

=> d his

```
     (FILE 'HOME' ENTERED AT 13:06:04 ON 15 OCT 2006)

     FILE 'USPATFULL' ENTERED AT 13:06:13 ON 15 OCT 2006
                E DOMS ROBERT W/IN
L1           12 S E3
                E HOXIE JAMES A/IN
L2            8 S E3
L3            4 S L2 NOT L1
                E BATES PAUL/IN
L4            6 S E3-E6
L5            5 S L4 NOT (L1 OR L2)
                E HOFFMAN TREVOR L/IN
L6            4 S E3
L7            0 S L6 NOT (L1 OR L2)
                E RUCKER JOSEPH/IN
L8            4 S E3-E5
L9            3 S L8 NOT (L1 OR L2)

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L11           6 S E3
L12           4 S L11 NOT L10

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L13         194 S E2-E4
L14         129 S L13 AND (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PA
L15           1 S L14 AND (SEVEN-SPANNING MEMBRANE OR MULTIPLE MEMBRANE OR MULT
L16          75 S L14 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID O
L17           0 S L16 AND (OPIOID OR OSPIN? OR BACTERIORHODOPSIN OR RHODOPSIN O
L18           0 S L16 AND (VIRUS-LIKE PARTICLE OR VLP OR VIRAL PSEUDOTYPE?)
L19          15 S L16 AND PY<1998

     FILE 'USPATFULL' ENTERED AT 13:36:57 ON 15 OCT 2006
L20        3910 S (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PARTICLE?
L21         678 S L20 AND (BACTERIORHODOPSIN OR RHODOPSIN OR OPSIN? OR PORIN? O
L22          17 S L21 AND (VLP?/CLM OR VIRUS-LIKE PARTICLE?/CLM OR RVVP?/CLM OR
L23           0 S L22 AND (BACTERIORHODOPSIN/CLM OR RHODOPSIN/CLM OR OPSIN?/CLM
L24         370 S L20 AND (VLP?/CLM OR VIRUS-LIKE PARTICLE?/CLM OR RVVP?/CLM OR
L25           6 S L24 AND (CCR5/CLM OR CXCR4/CLM OR MCAT?/CLM OR CXCR2/CLM OR C

=> file medline
COST IN U.S. DOLLARS                                 SINCE FILE      TOTAL
                                                          ENTRY    SESSION
FULL ESTIMATED COST                                       76.03     178.19

FILE 'MEDLINE' ENTERED AT 13:49:50 ON 15 OCT 2006

 FILE LAST UPDATED: 14 Oct 2006 (20061014/UP).  FILE COVERS 1950 TO DATE.

 On December 11, 2005, the 2006 MeSH terms were loaded.

 The MEDLINE reload for 2006 is now (26 Feb.) available.  For details
 on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>).
 See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

 OLDMEDLINE is covered back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2006 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> s (VLP? or virus-like particle? or RVVP? or retroviral virus like particle?)
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        420269 VIRUS
        385638 LIKE
        125929 PARTICLE?
          2766 VIRUS-LIKE PARTICLE?
                 (VIRUS(W)LIKE(W)PARTICLE?)
             2 RVVP?
         14948 RETROVIRAL
        420269 VIRUS
        385638 LIKE
```

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            1 RETROVIRAL VIRUS LIKE PARTICLE?
                (RETROVIRAL(W)VIRUS(W)LIKE(W)PARTICLE?)
L26       3320 (VLP? OR VIRUS-LIKE PARTICLE? OR RVVP? OR RETROVIRAL VIRUS LIKE
                PARTICLE?)

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          3147 CXCR4
           367 MCAT?
           532 CXCR2
           767 CXCR3
         49056 OPIOID
L27         33 L26 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID)

=> s 127 and py<1998
      11522178 PY<1998
                 (PY<19980000)
L28          3 L27 AND PY<1998

=> d 128,cbib,ab,1-3
```

L28 ANSWER 1 OF 3 MEDLINE on STN
94065898. PubMed ID: 8246048. Release of beta-endorphin and methionine-enkephalin into cerebrospinal fluid during deep brain stimulation for chronic pain. Effects of stimulation locus and site of sampling. Young R F; Bach F W; Van Norman A S; Yaksh T L. (Department of Neurological Surgery, University of California Irvine School of Medicine, Orange.) Journal of neurosurgery, **(1993 Dec)** Vol. 79, No. 6, pp. 816-25. Journal code: 0253357. ISSN: 0022-3085. Pub. country: United States. Language: English.

AB The authors systematically studied the release of the endogenous **opioid** peptides beta-endorphin and methionine (met)-enkephalin into the cerebrospinal fluid (CSF) during deep brain stimulation in patients suffering from otherwise intractable chronic pain. Nine patients were included in the study; six had stimulation electrodes placed in both the periventricular gray matter (PVG) and the thalamic nucleus ventralis posterolateralis (**VLP**) and three in the PVG only. Immunoreactivity of beta-endorphin and met-enkephalin (beta-EPir and MEir, respectively) was measured by radioimmunoassays in ventricular and lumbar CSF samples obtained before, during, and after stimulation. Prestimulation concentrations of beta-EPir and MEir were lower in ventricular than in lumbar CSF (6.6 +/- 0.5 vs. 13.7 +/- 1.0 pmol/liter, p = 0.0001, for beta-EPir; 33.6 +/- 5.1 vs. 48.3 +/- 3.2 pmol/liter, p < 0.05, for MEir). Ventricular CSF concentrations of both beta-EPir and MEir increased significantly during PVG stimulation, whereas VPL stimulation was without effect. No changes were seen in lumbar CSF levels of the peptides during stimulation in either site. A significant inverse relationship was found between the "during:before stimulation" ratios of visual analog scale ratings and beta-EPir levels during PVG stimulation. The beta-EPir and MEir concentration during:before stimulation ratios were positively correlated, whereas no correlation was present in prestimulation samples from ventricular or lumbar CSF. High-performance liquid chromatography of ventricular CSF pools obtained during PVG stimulation revealed that major portions of beta-EPir and MEir eluted as synthetic beta-endorphin and met-enkephalin, respectively, thus documenting the release of beta-endorphin and met-enkephalin into ventricular CSF during PVG stimulation. The finding of a direct relationship between beta-EPir release and pain alleviation may suggest a role for beta-endorphin in the analgesic mechanism of PVG stimulation.

L28 ANSWER 2 OF 3 MEDLINE on STN
87013273. PubMed ID: 2429096. Cardiovascular responses to medullary microinjections of opiate agonists in urethane-anesthetized rats. Punnen S; Sapru H N. Journal of cardiovascular pharmacology, **(1986 Sep-Oct)** Vol. 8, No. 5; pp. 950-6. Journal code: 7902492. ISSN: 0160-2446. Pub. country: United States. Language: English.

AB Pressor (**VLPA**) and depressor (VLDA) areas of the ventrolateral medulla were identified by microinjections of L-glutamate in urethane-anesthetized rats. Cardiovascular effects of opiate agonists microinjected into the same sites were then studied. Agents used to stimulate mu, delta, sigma, kappa, and beta-endorphin (epsilon) receptors were morphiceptin, D-Ala2-D-Leu5-enkephalin, N-allyl-normetazocine, dynorphin, and beta-endorphin, respectively. Opiate receptor stimulation in **VLPA** decreased blood pressure (BP) and heart rate (HR), while in VLDA it increased BP and HR. Thus, it is the site of injection rather than the type of opiate receptor that determines cardiovascular responses. Naloxone, an opiate antagonist, reversed and prevented these responses. Abolition of cardiovascular responses by spinal transection at the C1 level indicated that the sympathetic nervous system mediated these responses. The following mechanism is proposed for these actions of opiates: Cell bodies in **VLPA**, but not in VLDA, project to the intermediolateral cell column of the spinal cord. Opiates inhibit **VLPA**

inhibition of VLDA, which has an inhibitory effect on **VLPA**, results in an increase in BP and HR.

L28 ANSWER 3 OF 3 MEDLINE on STN
85112161. PubMed ID: 6097858. Contrasting effects of acute vs. chronic tricyclic antidepressant treatment on central morphine analgesia. Kellstein D E; Malseed R T; Goldstein F J. Pain, **(1984 Dec)** Vol. 20, No. 4, pp. 323-34. Journal code: 7508686. ISSN: 0304-3959. Pub. country: Netherlands. Language: English.

AB Antinociception following central **opioid** microinjection in rats was assessed weekly via a tail-flick procedure during chronic tricyclic antidepressant (TCA) treatment. (1) Daily TCA: Subcutaneous injections of desipramine (DMI), 30 mg/kg, chlorimipramine (CMI), 10 mg/kg, or saline, 1 ml/kg, were given daily for 22 days. Morphine sulfate (M), 5 micrograms, was microinjected into the ventrolateral periaqueductal gray (**VLPAG**) at 7 day intervals. On day 1, DMI or CMI enhanced M analgesia whereas saline did not. Augmentation of M disappeared by days 8 and 15 for CMI and DMI, respectively, and was replaced by attenuation which was still observed on day 22 for both TCAs. L-Tryptophan (LT), 100 mg/kg, i.p., on days 15 and 22 temporarily restored TCA enhancement of M. Fourteen days after cessation of all daily TCA treatments, enhancement of M by CMI was similar to that observed on day 1, whereas recovery of DMI-induced facilitation was incomplete. (2) Weekly TCA: Weekly treatment with DMI, CMI, or saline in the same doses as above had similar effects. M analgesia was enhanced by the TCAs but not saline on day 1; this facilitation was absent by day 15. Attenuation of M by DMI or CMI was evident on day 22; 2 weeks after cessation of all weekly TCA treatments, complete recovery of TCA-induced augmentation was observed. Loss of M facilitation during chronic daily or weekly TCA administration may be related to reduction of central **opioid** and/or 5-HT2 receptors.

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=> d his

     (FILE 'HOME' ENTERED AT 13:06:04 ON 15 OCT 2006)

     FILE 'USPATFULL' ENTERED AT 13:06:13 ON 15 OCT 2006
                E DOMS ROBERT W/IN
L1           12 S E3
                E HOXIE JAMES A/IN
L2            8 S E3
L3            4 S L2 NOT L1
                E BATES PAUL/IN
L4            6 S E3-E6
L5            5 S L4 NOT (L1 OR L2)
                E HOFFMAN TREVOR L/IN
L6            4 S E3
L7            0 S L6 NOT (L1 OR L2)
                E RUCKER JOSEPH/IN
L8            4 S E3-E5
L9            3 S L8 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 13:10:28 ON 15 OCT 2006
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L10           7 S E1-E2
                E HOXIE J A/IN
L11           6 S E3
L12           4 S L11 NOT L10

     FILE 'MEDLINE' ENTERED AT 13:12:03 ON 15 OCT 2006
                E DOMS R W/AU
L13         194 S E2-E4
L14         129 S L13 AND (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PA
L15           1 S L14 AND (SEVEN-SPANNING MEMBRANE OR MULTIPLE MEMBRANE OR MULT
L16          75 S L14 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID O
L17           0 S L16 AND (OPIOID OR OSPIN? OR BACTERIORHODOPSIN OR RHODOPSIN O
L18           0 S L16 AND (VIRUS-LIKE PARTICLE OR VLP OR VIRAL PSEUDOTYPE?)
L19          15 S L16 AND PY<1998

     FILE 'USPATFULL' ENTERED AT 13:36:57 ON 15 OCT 2006
L20        3910 S (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PARTICLE?
L21         678 S L20 AND (BACTERIORHODOPSIN OR RHODOPSIN OR OPSIN? OR PORIN? O
L22          17 S L21 AND (VLP?/CLM OR VIRUS-LIKE PARTICLE?/CLM OR RVVP?/CLM OR
L23           0 S L22 AND (BACTERIORHODOPSIN/CLM OR RHODOPSIN/CLM OR OPSIN?/CLM
L24         370 S L20 AND (VLP?/CLM OR VIRUS-LIKE PARTICLE?/CLM OR RVVP?/CLM OR
L25           6 S L24 AND (CCR5/CLM OR CXCR4/CLM OR MCAT?/CLM OR CXCR2/CLM OR C

     FILE 'MEDLINE' ENTERED AT 13:49:50 ON 15 OCT 2006
L26        3320 S (VLP? OR VIRUS-LIKE PARTICLE? OR RVVP? OR RETROVIRAL VIRUS LI
L27          33 S L26 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID)
L28           3 S L27 AND PY<1998
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         14948 RETROVIRAL
        122993 VECTOR?
          4482 RETROVIRAL VECTOR?
                 (RETROVIRAL(W)VECTOR?)
          1662 LENTIVIRAL
         65832 VECTOR
           564 LENTIVIRAL VECTOR
                 (LENTIVIRAL(W)VECTOR)
L29       4997 (RETROVIRAL VECTOR? OR LENTIVIRAL VECTOR)

=> s 129 and (CCR5 or CXCR4 or MCAT? or CXCR2 or CXCR3 or opioid)
          3836 CCR5
          3147 CXCR4
           367 MCAT?
           532 CXCR2
           767 CXCR3
         49056 OPIOID
L30         43 L29 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID)

=> s 130 and py<1998
      11522178 PY<1998
                 (PY<19980000)
L31          1 L30 AND PY<1998

=> d 131,cbib,ab

L31  ANSWER 1 OF 1     MEDLINE on STN
1998035784.    PubMed ID: 9367958.   Targeting of HIV- and SIV-infected cells
     by CD4-chemokine receptor pseudotypes. Endres M J; Jaffer S; Haggarty B;
     Turner J D; Doranz B J; O'Brien P J; Kolson D L; Hoxie J A. (Department of
     Medicine, Hematology-Oncology Division, University of Pennsylvania,
     Philadelphia, PA 19104, USA.. endres@mail.med.upenn.edu) . Science, (1997
     Nov 21) Vol. 278, No. 5342, pp. 1462-4.  Journal code: 0404511. ISSN:
     0036-8075. Pub. country: United States. Language: English.
AB   Retroviral vectors containing CD4 and an appropriate chemokine
     receptor were evaluated for the ability to transduce cells infected with
     human immunodeficiency virus (HIV) and simian immunodeficiency virus
     (SIV).  These CD4-chemokine receptor pseudotypes were able to target HIV-
     and SIV-infected cell lines and monocyte-derived macrophages in a manner
     that corresponded to the specificity of the viral envelope glycoprotein
     for its CD4-chemokine receptor complex.  This approach could offer a way
     to deliver antiviral genes directly to HIV-infected cells in vivo and
     could provide an additional treatment strategy in conjunction with
     existing antiviral therapies.


=> d his

     (FILE 'HOME' ENTERED AT 13:06:04 ON 15 OCT 2006)

     FILE 'USPATFULL' ENTERED AT 13:06:13 ON 15 OCT 2006
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L2            8 S E3
L3            4 S L2 NOT L1
                E BATES PAUL/IN
L4            6 S E3-E6
L5            5 S L4 NOT (L1 OR L2)
                E HOFFMAN TREVOR L/IN
L6            4 S E3
L7            0 S L6 NOT (L1 OR L2)
                E RUCKER JOSEPH/IN
L8            4 S E3-E5
L9            3 S L8 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 13:10:28 ON 15 OCT 2006
                E DOMS ROBERT W/IN
L10           7 S E1-E2
                E HOXIE J A/IN
L11           6 S E3
L12           4 S L11 NOT L10

     FILE 'MEDLINE' ENTERED AT 13:12:03 ON 15 OCT 2006
                E DOMS R W/AU
L13         194 S E2-E4
L14         129 S L13 AND (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PA
L15           1 S L14 AND (SEVEN-SPANNING MEMBRANE OR MULTIPLE MEMBRANE OR MULT
L16          75 S L14 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID O
L17           0 S L16 AND (OPIOID OR OSPIN? OR BACTERIORHODOPSIN OR RHODOPSIN O
L18           0 S L16 AND (VIRUS-LIKE PARTICLE OR VLP OR VIRAL PSEUDOTYPE?)
L19          15 S L16 AND PY<1998
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     FILE 'USPATFULL' ENTERED AT 13:36:57 ON 15 OCT 2006
L20        3910 S (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PARTICLE?
L21         678 S L20 AND (BACTERIORHODOPSIN OR RHODOPSIN OR OPSIN? OR PORIN? O
L22          17 S L21 AND (VLP?/CLM OR VIRUS-LIKE PARTICLE?/CLM OR RVVP?/CLM OR
L23           0 S L22 AND (BACTERIORHODOPSIN/CLM OR RHODOPSIN/CLM OR OPSIN?/CLM
L24         370 S L20 AND (VLP?/CLM OR VIRUS-LIKE PARTICLE?/CLM OR RVVP?/CLM OR
L25           6 S L24 AND (CCR5/CLM OR CXCR4/CLM OR MCAT?/CLM OR CXCR2/CLM OR C

     FILE 'MEDLINE' ENTERED AT 13:49:50 ON 15 OCT 2006
L26        3320 S (VLP? OR VIRUS-LIKE PARTICLE? OR RVVP? OR RETROVIRAL VIRUS LI
L27          33 S L26 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID)
L28           3 S L27 AND PY<1998
L29        4997 S (RETROVIRAL VECTOR? OR LENTIVIRAL VECTOR)
L30          43 S L29 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID)
L31           1 S L30 AND PY<1998

=> s l29 and (bacteriorhodopsin or rhodopsin or opsin? or adrenergic or seven-spanning membrane or multiple transmembra
          2390 BACTERIORHODOPSIN
          6435 RHODOPSIN
          2249 OPSIN?
        111660 ADRENERGIC
        276904 SEVEN
         17217 SPANNING
        666997 MEMBRANE
             2 SEVEN-SPANNING MEMBRANE
                 (SEVEN(W)SPANNING(W)MEMBRANE)
        490465 MULTIPLE
         49181 TRANSMEMBRANE
           110 MULTIPLE TRANSMEMBRANE
                 (MULTIPLE(W)TRANSMEMBRANE)
L32          17 L29 AND (BACTERIORHODOPSIN OR RHODOPSIN OR OPSIN? OR ADRENERGIC
                OR SEVEN-SPANNING MEMBRANE OR MULTIPLE TRANSMEMBRANE)

=> s l32 and py<1998
      11522178 PY<1998
                 (PY<19980000)
L33           8 L32 AND PY<1998

=> d l33,cbib,ab,1-8
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L33 ANSWER 1 OF 8 MEDLINE on STN
1998050928. PubMed ID: 9390562. Crx, a novel otx-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. Furukawa T; Morrow E M; Cepko C L. (Department of Genetics, and Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts 02115, USA.) Cell, **(1997 Nov 14)** Vol. 91, No. 4, pp. 531-41. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB We have isolated a novel otx-like homeobox gene, Crx, from the mouse retina. Crx expression is restricted to developing and mature photoreceptor cells. CRX bound and transactivated the sequence TAATCC/A, which is found upstream of several photoreceptor-specific genes, including the **opsin** genes from many species. Overexpression of Crx using a **retroviral vector** increased the frequency of clones containing exclusively rod photoreceptors and reduced the frequency of clones containing amacrine interneurons and Muller glial cells. In addition, presumptive photoreceptor cells expressing a dominant-negative form of CRX failed to form proper photoreceptor outer segments and terminals. Crx is a novel photoreceptor-specific transcription factor and plays a crucial role in the differentiation of photoreceptor cells.

L33 ANSWER 2 OF 8 MEDLINE on STN
1998023747. PubMed ID: 9358855. Retrovirus-mediated gene transfer into rat salivary gland cells in vitro and in vivo. Barka T; van der Noen H M. (Department of Cell Biology and Anatomy, Mount Sinai School of Medicine, City University of New York, New York 10029, USA.) The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, **(1997 Nov)** Vol. 45, No. 11, pp. 1533-45. Journal code: 9815334. ISSN: 0022-1554. Pub. country: United States. Language: English.

AB A **retroviral vector** DAP that encodes the human placental alkaline phosphatase (PLAP) and the neomycin-resistant gene was used to transduce the salivary gland-derived cell line A5 in vitro and acinar cells in rat submandibular gland in vivo. Expression of the transduced PLAP gene was established by histochemical staining for heat-resistant AP and by determination of enzyme activity. From the in vitro experiments, we concluded that the salivary gland-derived cell line A5 can be infected by the **retroviral vector** DAP. In the transduced cells the viral long terminal repeat (LTR) promoter was effective, and the cells expressed heat-stable PLAP which was localized mostly in the plasma membrane and could be released by treatment with bromelain or phosphatidyinositol-specific phospholipase C. A5-DAP cells secreted PLAP into the medium. Clones of A5-DAP cells expressed various levels of the enzyme. The level

Retrograde ductal injection of the viral vector into the duct of the submandibular gland of rats resulted in integration and long-term expression of PLAP gene in acinar cells. Expression of PLAP was seen up to 25 days, the limit of the observation period. To facilitate integration of the viral DNA, cell division of acinar cells was induced by administration of the beta-**adrenergic** agonist isoproterenol before administration of the virus. PLAP was secreted into submandibular saliva. The data support the notion that salivary glands are suitable targets for gene transfer in vivo by a **retroviral vector**.

L33 ANSWER 3 OF 8 MEDLINE on STN

97439862. PubMed ID: 9294208. Stable and efficient gene transfer into the retina using an HIV-based **lentiviral vector**. Miyoshi H; Takahashi M; Gage F H; Verma I M. (Laboratory of Genetics, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA.) Proceedings of the National Academy of Sciences of the United States of America, **(1997 Sep 16)** Vol. 94, No. 19, pp. 10319-23. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The development of methods for efficient gene transfer to terminally differentiated retinal cells is important to study the function of the retina as well as for gene therapy of retinal diseases. We have developed a **lentiviral vector** system based on the HIV that can transduce terminally differentiated neurons of the brain in vivo. In this study, we have evaluated the ability of HIV vectors to transfer genes into retinal cells. An HIV vector containing a gene encoding the green fluorescent protein (GFP) was injected into the subretinal space of rat eyes. The GFP gene under the control of the cytomegalovirus promoter was efficiently expressed in both photoreceptor cells and retinal pigment epithelium. However, the use of the **rhodopsin** promoter resulted in expression predominantly in photoreceptor cells. Most successfully transduced eyes showed that photoreceptor cells in >80% of the area of whole retina expressed the GFP. The GFP expression persisted for at least 12 weeks with no apparent decrease. The efficient gene transfer into photoreceptor cells by HIV vectors will be useful for gene therapy of retinal diseases such as retinitis pigmentosa.

L33 ANSWER 4 OF 8 MEDLINE on STN

97079515. PubMed ID: 8921226. Use of a **retroviral vector** with an internal **opsin** promoter to direct gene expression to retinal photoreceptor cells. Kido M; Rich K A; Yang G; Barron E; Kohn D B; al-Ubaidi M R; Blanks J C; Lang G. (Doheny Eye Institute Department of Ophthalmology, USC School of Medicine, Los Angeles, CA, USA.) Current eye research, **(1996 Aug)** Vol. 15, No. 8, pp. 833-44. Journal code: 8104312. ISSN: 0271-3683. Pub. country: ENGLAND: United Kingdom. Language: English.

AB PURPOSE: Viral-mediated gene transfer to retina, as well as to other tissues, is evolving rapidly. We have evaluated the potential of a **retroviral vector** with an internal **opsin** promoter fragment to direct gene expression to retinal photoreceptor cells. METHODS: Two recombinant **retroviral vectors** were prepared; in each Vector, a 1.4 kb fragment of the mouse **opsin** promoter was placed downstream from the neoR gene in the Moloney murine leukemia virus-based vector G1Na. The **opsin** promoter fragment was linked either to the cDNA for mouse rod photoreceptor phosphodiesterase (PDE) beta-subunit or to the bacterial lacZ reporter gene. These vectors were tested for their ability to direct gene expression after transduction of 3T3 and Y79 cells, or of dissociated retinal cell cultures or retinal explants from neonatal mice. RESULTS: As expected, PDE beta-subunit and beta-galactosidase mRNAs were expressed only at low levels in 3T3 fibroblasts and Y79 retinoblastoma cells. Northern blot analysis indicated that expression was derived from the viral long terminal repeat (LTR) promoter. Infection of primary retinal cell cultures or explants from neonatal mice with BAG retrovirus, in which beta-galactosidase is driven by the viral LTR, resulted in expression in many cell types, while the **opsin**-lacZ vector mediated the expression of the lacZ reporter gene specifically in photoreceptor cells. CONCLUSIONS: The internal **opsin** promoter fragment appears capable of selectively directing gene expression to photoreceptor cells after retroviral-mediated gene transfer. These findings serve as a basis for future studies using the **opsin** promoter-beta PDE **retroviral vector** to rescue photoreceptor cells in the rd mutant mouse, in which the beta-PDE gene is mutated resulting in degeneration of photoreceptor cells during the early postnatal period.

L33 ANSWER 5 OF 8 MEDLINE on STN

96243951. PubMed ID: 8845386. Retrovirus-mediated gene transfer into salivary glands in vivo. Barka T; Van der Noen H M. (Department of Cell Biology and Anatomy, Mount Sinai School of Medicine, City University of New York, NY 10029, USA.) Human gene therapy, **(1996 Mar 20)** Vol. 7, No. 5, pp. 613-8. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB In the present report, we show prolonged expression of beta-galactosidase (beta-Gal) in the acinar cells of the submandibular and sublingual glands

BAG. To facilitate integration of viral DNA, cell division in the gland was induced by the administration of the beta-**adrenergic** agonist isoproterenol prior to the delivery of the vector. The frequency of cells stained for beta-Gal was higher if the virus was injected 4-20 hr after the two injections of isoproterenol given 24 hr apart than after the injection of only one dose of the drug. Without stimulation of cell division, no integration of the viral DNA was observed. Expression of the marker enzyme was observed up to 43 days, the limit of the observation period. The data indicate that salivary glands are potential targets of retrovirus-mediated gene transfer for somatic gene therapy.

L33 ANSWER 6 OF 8 MEDLINE on STN
92200199. PubMed ID: 1686984. The use of genetically altered astrocytes to provide nerve growth factor to adrenal chromaffin cells grafted into the striatum. Cunningham L A; Hansen J T; Short M P; Bohn M C. (Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, NY 14642.) Brain research, **(1991 Oct 11)** Vol. 561, No. 2, pp. 192-202. Journal code: 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

AB Transplantation of adrenal chromaffin cells into the striatum of Parkinson's disease patients is unlikely to become a reliable therapy unless techniques are devised to improve cell survival. To address this issue, we investigated the use of genetically altered astrocytes that constitutively secrete beta-nerve growth factor (NGF) to provide trophic support for adrenal chromaffin cells grafted into the dopamine-denervated striatum of the rat. Primary rat astrocytes were altered genetically in vitro by infection with a **retroviral vector** harboring a mouse beta-NGF transgene under constitutive long terminal repeat transcriptional control. Confluent cultures of these genetically altered astrocytes secrete NGF into their culture medium at a rate of approximately 9 pg/10(5) cells/h. This rate of NGF secretion is at least 10-fold higher than that of confluent sister cultures of uninfected astrocytes. The effects of the NGF-secreting astrocytes on the survival and neuronal transformation of dissociated adrenal chromaffin cells were assessed in vitro and following transplantation into the dopamine-denervated striatum of the adult rat. In vitro experiments demonstrated that neuritic outgrowth is stimulated when postnatal day 12 chromaffin cells are grown on a monolayer of the genetically altered astrocytes. When co-grafted with genetically altered astrocytes, young postnatal chromaffin cells displayed extensive neuritic outgrowth within the host brain 2 weeks postimplantation, whereas chromaffin cells grafted alone or with normal astrocytes retain an endocrine-like morphology. Survival of the chromaffin cells is also enhanced 3-6-fold when co-grafted with the genetically altered astrocytes. In addition, the neuronally transformed chromaffin cells appear to lose **adrenergic** properties as assessed by diminished immunoreactivity to the **adrenergic** marker, phenylethanolamine-N-methyltransferase. Although their survival is also enhanced approximately 4-fold relative to controls, adult chromaffin cells do not convert to a neuronal morphology when co-grafted with the genetically altered astrocytes. These studies demonstrate that rat astrocytes carrying a mouse NGF transgene provide trophic support for intrastriatal chromaffin cell grafts.

L33 ANSWER 7 OF 8 MEDLINE on STN
90222170. PubMed ID: 2158101. Immortalization of bipotential and plastic glio-neuronal precursor cells. Evrard C; Borde I; Marin P; Galiana E; Premont J; Gros F; Rouget P. (Laboratoire de Biochimie Cellulaire, College de France, Paris.) Proceedings of the National Academy of Sciences of the United States of America, **(1990 Apr)** Vol. 87, No. 8, pp. 3062-6. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Permanent clonal cell lines from newborn mouse striatum have been established after transfer of the simian virus 40 large tumor oncogene by means of a **retroviral vector**. Some of the lines obtained displayed properties of bipotential and plastic glio-neuronal precursors. Depending on the culture conditions, these cells express either the glial fibrillary acidic protein or neurofilaments. In addition, the cells can display **adrenergic**, D1 and D2 dopaminergic, muscarinic, and 5-hydroxytryptamine type 2 serotoninergic receptors, which are coupled either to the adenylate cyclase or to the phosphatidylinositol signaling pathways. The panel of receptors for neurotransmitters exhibited by these lines closely resembles that of primary striatal neurons. Results suggest that plastic common precursors of astrocytes and neurons persist in the striatum at a late developmental stage. As these permanent cell lines constitute an unlimited source of homogenous cell material, we suggest that they should be useful for molecular and pharmacological studies on the mechanisms and regulation of signal transduction as well as the commitment, plasticity, and differentiation of neural cells.

L33 ANSWER 8 OF 8 MEDLINE on STN
88277894. PubMed ID: 2899356. Carboxyl terminal domain of Gs alpha specifies coupling of receptors to stimulation of adenylyl cyclase. Masters S B; Sullivan K A; Miller R T; Beiderman B; Lopez N G;

California, San Francisco 94143.) Science, **(1988 Jul 22)** Vol. 241, No. 4864, pp. 448-51. Journal code: 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB The alpha subunits of Gs and Gi link different sets of hormone receptors to stimulation and inhibition, respectively, of adenylyl cyclase. A chimeric alpha i/alpha s cDNA was constructed that encodes a polypeptide composed of the amino terminal 60% of an alpha i chain and the carboxyl terminal 40% of alpha s. The cDNA was introduced via a **retroviral vector** into S49 cyc- cells, which lack endogenous alpha s. Although less than half of the hybrid alpha chain is derived from alpha s, its ability to mediate beta-adrenoceptor stimulation of adenylyl cyclase matched that of the normal alpha s polypeptide expressed from the same **retroviral vector** in cyc- cells. This result indicates that carboxyl terminal amino acid sequences of alpha s contain the structural features that are required for specificity of interactions with the effector enzyme, adenylyl cyclase, as well as with the hormone receptor.

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=> d his

     (FILE 'HOME' ENTERED AT 13:06:04 ON 15 OCT 2006)

     FILE 'USPATFULL' ENTERED AT 13:06:13 ON 15 OCT 2006
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L2            8 S E3
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L4            6 S E3-E6
L5            5 S L4 NOT (L1 OR L2)
                E HOFFMAN TREVOR L/IN
L6            4 S E3
L7            0 S L6 NOT (L1 OR L2)
                E RUCKER JOSEPH/IN
L8            4 S E3-E5
L9            3 S L8 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 13:10:28 ON 15 OCT 2006
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                E HOXIE J A/IN
L11           6 S E3
L12           4 S L11 NOT L10

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L13         194 S E2-E4
L14         129 S L13 AND (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PA
L15           1 S L14 AND (SEVEN-SPANNING MEMBRANE OR MULTIPLE MEMBRANE OR MULT
L16          75 S L14 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID O
L17           0 S L16 AND (OPIOID OR OSPIN? OR BACTERIORHODOPSIN OR RHODOPSIN O
L18           0 S L16 AND (VIRUS-LIKE PARTICLE OR VLP OR VIRAL PSEUDOTYPE?)
L19          15 S L16 AND PY<1998

     FILE 'USPATFULL' ENTERED AT 13:36:57 ON 15 OCT 2006
L20        3910 S (VIRUS-LIKE PARTICLE? OR VLP? OR RETROVIRAL VECTOR PARTICLE?
L21         678 S L20 AND (BACTERIORHODOPSIN OR RHODOPSIN OR OPSIN? OR PORIN? O
L22          17 S L21 AND (VLP?/CLM OR VIRUS-LIKE PARTICLE?/CLM OR RVVP?/CLM OR
L23           0 S L22 AND (BACTERIORHODOPSIN/CLM OR RHODOPSIN/CLM OR OPSIN?/CLM
L24         370 S L20 AND (VLP?/CLM OR VIRUS-LIKE PARTICLE?/CLM OR RVVP?/CLM OR
L25           6 S L24 AND (CCR5/CLM OR CXCR4/CLM OR MCAT?/CLM OR CXCR2/CLM OR C

     FILE 'MEDLINE' ENTERED AT 13:49:50 ON 15 OCT 2006
L26        3320 S (VLP? OR VIRUS-LIKE PARTICLE? OR RVVP? OR RETROVIRAL VIRUS LI
L27          33 S L26 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID)
L28           3 S L27 AND PY<1998
L29        4997 S (RETROVIRAL VECTOR? OR LENTIVIRAL VECTOR)
L30          43 S L29 AND (CCR5 OR CXCR4 OR MCAT? OR CXCR2 OR CXCR3 OR OPIOID)
L31           1 S L30 AND PY<1998
L32          17 S L29 AND (BACTERIORHODOPSIN OR RHODOPSIN OR OPSIN? OR ADRENERG
L33           8 S L32 AND PY<1998

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
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